STUDIES ON ENDOMYCORRHIZAL ASSOCIATIONS OF AROMATIC *CYMBOPOGON* SPECIES IN RELATION TO THEIR GROWTH AND PRODUCTIVITY

ABSTRACT

Thesis submitted for the award of the degree of

Doctor of Philosophy

IN

BOTANY

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DEPARTMENT OF BOTANY
ALIGARH MUSLIM UNIVERSITY
ALIGARH (INDIA)

1998

ABSTRACT

The *Cymbopogon* Spreng. is a genus of perennial grasses which belongs to the family Poaceae. Most of the species are aromatic and some of them yeild essential oils of commercial importance. These oils are intensively used in perfumeries, cosmetics and pharmaceautical preparations. *Cymbopogon martinii* (Roxb.) Wats with two varieties, *motia* for the production of palmarosa oil and *sofia* for ginger-grass oil, *C. flexuosus* (Steud.) Wats., *C. citratus* Stapf. and *C. pendulus* Stapf. for lemon-grass oil, *C. winterianus* Jowitt. and *C. nardus* (L.) Rendle for citronella oil are six most commercially exploited species throughout the world. But in India, *C. martinii*, *C. flexuosus* and *C. winterianus* are the three major species under large scale of commercial cultivation followed by *C. citratus*, *C. pendulus* and *C. nardus*.

The crushed leaves of these *Cymbopogon* spp. give off different types of fragrances due to the presence of essential oils containing a variety of aromatic compounds. Main constituents of the essential oils of some important *Cymbopogon* spp. are geraniol, citral, citronellal, citronellol and piperitone. These essential oils also contain a large number of minor constituents. The oil of *C. martinii* (palmarosa oil) is used as a source of geranium odour because true geranium oil is more expensive. Large quantity of oil is exported for use in perfumery. Besides its perfumery value, palmarosa oil has antiseptic properties and some medicinal importance. East Indian lemon-grass (*C. flexuosus*) oil is used specially for the extraction of citral, a starting material

for the preparation of ionones (α-inonone and β-ionone). The α-ionone is used in flavours, cosmetics and perfumes where as β- ionone is used in manufacture of synthetic vitamin A. *C. winterianus* yields Java citronella oil which is used in perfumary, soap, cosmetic and pharmaceutical industries, also as mosquito repellent and potential germicide.

Cymbopogon industry in India has vast and expanding business potentials. To meet the growing demand of essential oils, several high yielding varieties have been developed as a result of research and the research is still in progress to increase the yield of plants.

Mycorrhizae have been found to play an important role in increasing the plants growth. Mycorrhizae are mutualistic associations between plant roots and fungi which occur naturally throughout the terrestrial ecosystem. Among various forms of mycorrhizae, vesicular-arbuscular mycorrhizal are the commonest and wide spread, occurring in association of large number of species. VAM fungi cause favourable effect on plant growth, and improve nutrient and water uptake. A perusal of available literature shows that some VAM fungi remain associated with *Cymbopogon* spp. The identity of such VAM fungi has not been established and investigation on the mycorrhizal associations of *Cymbopogon* species is meager. Keeping in view the inadequalcy of the information on *Cymbopogon*- VAM associations the present investigations were undertaken.

All the five *Cymbopogon* spp. namely *C. caesius, C. flexuosus, C. maritnii, C. pendulus* and *C. winterianus* grown in the field at the CIMAP, Lucknow exhibited VAM association. The degree of colonization, however,

varied. In order to determine seasonal variation, plants were examined periodically at monthly intervals for one year (June '92 to May '93). Seasonal variation in the VAM colonization and spore count were noticed. The VAM fungi associated with palmarosa roots also occurred in the tissues inside vascular cylinder including large metaxylem and pith cells. Population density of spores in the rhizosphere was not found to be correlated to the degree of root colonization. The present study showed that abundant VAM spores were present in the rhizosphere of *Cymbopogon* spp. indicating that the selected plants invariably develop mycorrhizal association in nature. *Glomus* was observed to be predominant genus. Nine, species of *Glomus* namely, *G. aggregatum*, *G. dimorphicum*, *G. fasciculatum*, *G. geosporum*, *G. macrocarpum*, *G. mosseae*, *G. multicaulis*, *G. occultum*, *G. reticulatum* and also one *Gigaspora* sp. were found associated with species of *Cymbopogon*. The association of *G. dimorphicum*, *G. fasciculatum*, *G. geosporum*, *G. macrocarpum*, *G. mosseae*, *G. multicaulis*, *G. occultum*, *G. reticulatum* and *Gigaspora* sp. with *Cymbopogon* spp. is reported for the first time.

Multiplication of the VAM fungi found associated with the *Cymbopogon* spp. was affected by the weather conditions. Spore count was low in summer months, tended to increase in high relative humidity and moderate temperature, remained almost stable during winters. Higest spore count was observed in February and March. Water logging abversly affected the VAM spore counts. VAM spore number and the extent of root colonization showed no correlation. Highest root infection was found in summer when temperature was high and humidity low.

Glasshouse experiments were carried out to determine effect of inoculation of *Cymbopogon* spp. by VAM fungi on growth performance, oil yeild, uptake of nutrients from soil and their accumulation in shoot tissues. Three commercially important species of *Cymbopogon* viz *C. flexuosus, C. martinii* and *C. winterianus* and VAM fungi *G. aggregatum, G. fasciculatum* and *G. mosseae* were selected. For inoculation, the VAM species were used singly and in mixture of all the three VAM fungi.

All the inoculated plants showed a significant increase in their plant height, tillering and shoot fresh weight in comparison to control plants. Mycorrhization also improved essential oil production by the *Cymbopogon* spp. The increase in essential oil production showed significant improvement when increase in the total fresh herb yield of the plants was taken into account. The symbiotic association, however, did not affect the main coustituents of essential oils. Nutrient uptake and their accumulation in shoot tissues considerably increased as a results of mycorrhizal association. An increasae in nutrient (N,P,K, Cu and Zn) uptake and their accumulation was greater in all the species of *Cymbopogon* irrespective of the *Glomus* spp. involved. Enhancement in the accumulation of N,P,K,Cu and Zn in shoot tissues was in consistence with the biomass increase.

Cymbopogon winterianus showed best response to inoculation in all the considered parameters in comparison to other two *Cymbopogon* species. Among the VAM treatements, single inoculation with *G. aggregatum* and *G. fasciculatum* proved to be better than *G. mosseae* and mixture of all the three species.

Results of the investigation show that vesicular-arbuscular mycorrhizae (VAM fungi) have potential to enhance the plant growth, plant productivity and essential oil content by improving nutritional status of the *Cymbopogon* spp. This potential can be exploited for commercial benefits by applying VAM fungi artificially in order to enhance the essential oil production by *Cymbopogon* spp.

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In the name of Allah
The most Beneficent ,
The Merciful

وَالْاَرْضَ وَضَعَهَا لِلاَنَامِ ○
فِيْهَا فَاكِهَةٌ وَّ النَّخْلُ ذَاتُ الْاَكْمَامِ ○
وَ الْحَبُّ ذُو الْعَصْفِ وَالرَّيْحَانُ ○
فَبِاَیِّ اٰلَآءِ رَبِّكُمَا تُكَذِّبٰنِ ○

(القرآن ۵۵: ۱۰-۱۳)

And the earth hath He appointed for (His) creatures,

Wherein are fruit and sheathed palm-trees,

Husked grain and scented herb.

Which is it, of the favours of your Lord, that ye deny?

(Al-Quran,55:10-13)

Dedicated

to my

loving Father and Mother

Mr. Mohammad Ahmad Farooqui

Mrs. Rabia Naseem Farooqui

Dated : 30.07.98

CERTIFICATE

This is to certify that the thesis entitled **"Studies on the endomycorrhizal associations of aromatic *Cymbopogon* species in relation to their growth and productivity"** submitted to the Aligarh Muslim University for the award of the degree of **Doctor of Philosophy** embodies the bonafide and original work of **Ms Fauzia Naushin** carried out under our joint supervision and that no part of this work has been submitted for any other degree or diploma.

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ACKNOWLEDGEMENTS

It gives me immense pleasure to express my sincere gratitude to my internal supervisor **Prof. M. Wajid Khan**, Chairman, Department of Botany, Aligarh Muslim University, Aligarh, for his valuable guidance. He had been a source of inspiration and encoragement throughout the course of the present study. Prof. M. Wajid Khan, took keen interst and spared enough time out of his busy schedule to supervise the work.

I am extremely indebted to my external supervisior **Dr. K.K. Janardhanan** Scientist Emeritus and formerly Assistant Director and Head, Division of Plant Pathology, Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow, for suggesting the problem, bountiful suggestion, and motivation during the experimental phase of this work at CIMAP, Lucknow.

I am thankful to Dr. Sushil Kumar, The Director, CIMAP, Lucknow for providing all necessary facilities during experimental work. I am also thankful to Prof. M. Wajid Khan, Chairman, Department of Botany and Prof. Wazahat Husain and Prof. Saeed A. Siddiqui former Chairmen, Department of Botany, A.M.U., Aligarh for providing necessary facilities during the chemical analysis.

I am also thankful to Dr. H.S. Rathore, Chairman, Department of Applied Chemistry, Z.H. College of Engineering and Technology, A.M.U., Aligarh and Dr. R.A.K. Rao, Lecturer and Incharge of Instrumentation Laboratory, Department of Applied Chemistry, Z.H. College of Engineering and Technology, A.M.U., Aligarh for their help and cooperation in the analysis of micronutrients.

I wish to thank Dr. S. Mandal, CIMAP, Lucknow for his assistance in gas liquid chromatography of the oil.

Well deserved credit goes to my husband Dr. Fareed A. Khan, Lecturer, Department of Botany, A.M.U., Aligarh for anatomical details, statistical analysis, constant help, cooperation and suggestions throughout the course of the study.

I express my sincere thanks to Dr. S. Kumar, Head, Division of Plant Pathology, CIMAP, Lucknow, Dr. Mansoor Alam and Dr. M.L. Gupta Scientists, Division of Plant Pathology, CIMAP, Lucknow for their constant help, suggestions, encouragement and cooperation throghout the work. My thanks are also due to Dr. A. Haseeb, Dr. A. Sattar, Dr. A. Samad, Dr. M. Zaim Scientists, CIMAP, Lucknow for their cooperation and help. Constant help, cooperation and suggestions of my senior laboratory colleagues Dr. Abdul Khaliq, Dr. Farzana Butool and Dr. Neelima Ratti are also thankfully acknowledged.

Thanks are also due to Dr. Athar A. Khan, Dr. M. Masroor A. Khan and Dr. Abrar A. Khan, Lecturers, Department of Botany, A.M.U., Aligarh for their help and suggestions. I am also thankful to Dr. Yasmeen, Dr. Kamal Singh, Dr. Badshah Khan, Ms. Shazia Siddiqui and Ms. Ira Upadhyay for their cooperation and help.

Last but not the least my heartiest thanks are due to Ms. Shazia Naurin and Dr. Nazia Seemin, my sisters for their continuous cooperation and moral support at every step during the period of this invistegation and ever willing help when needed.

(FAUZIA NAUSHIN)

CONTENTS

INTRODUCTION

The genus *Cymbopogon* Spreng. of the family Poaceae includes many economically important species. Most of the species are aromatic and some of them yield essential oils of commercial importance. The species of *Cymbopogon* largely cultivated in different parts of the world are *C. martinii* (Roxb.) Wats. with two varieties Motia, and Sofia. *C.flexuosus* (Steud.) Wats., *C.citratus* Stapf., *C.pendulus* Stapf. and *C.khasianus* (Hack.) Stapf ex Bor, *C. winterianus*, Jowitt and *C. nardus* (L.) Rendle. But in India, *C.martinii*, *C.flexuosus* and *C.winterianus* Jowitt. are the three major species under large scale of commercial cultivation followed by the *C.citratus*, *C.pendulus* and *C.nardus*.

Cymbopogon martinii is commonly known as Rosha or Rusha-grass and occurs in two morphologically indistinguishable varieties, Motia and Sofia. The former yields palmarosa oil and the latter ginger-grass oil. *C.martinii* is a native of India and is mainly cultivated in Madhya Pradesh, Maharashtra and Assam. The bulk of palmarosa oil is extracted and exported from India.

Cymbopogon flexuosus commonly known as East Indian lemon-grass, yields East Indian lemon-grass oil. It originated in India and is mainly distributed in Tirunnelveli, Travencore and Cochin in Kerala. *C.citratus* is called West Indian lemon-grass which originated either in India or Indonesia (Maheshwari, 1966; Strauss, 1969). *C.pendulus* is a native of India and

commonly known as Jammu lemon-grass as it occurs in sub-Himalayan regions and has been successfully cultivated in Jammu and hilly areas of Kangra (Palampur) in Himachal Pradesh in India.

Cymbopogon winterianus a native of Sri Lanka is the primary source of citronella oil. It is cultivated on large scale in Indonesia (Java) as principal aromatic grass, hence named 'Java citronella'. The main producers of Java citronella oil are Farmosa (Taiwan), China and Indonesia. In India, it is particularly grown in Assam, West Bengal and Uttar Pradesh and also to some extent in Maharashtra, Goa, Daman, Diu, Gujarat, Karnataka, Andhra Pradesh and Tamil Nadu.*C.nardus* known as Ceylon citronella is indigenous to Sri Lanka. *Cymbopogon jwarancusa* (Jones) Schult. is indigenous to India occurring in Himalayas, i.e., from Kashmir to Assam and in North Western Plains down to Bombay (Guenther, 1950; Anonymous, 1950; Chopra *et al.*,1956). *Cymbopogon caesius* (Nees) Stapf. commonly known as Kachi-grass occurs in Madras, Mysore, Travancore and Gujarat in India and also in Arabia and Somalia.

The bruised leaves of these *Cymbopogon* spp. give off different types of fragrance due to the presence of essential oils containing a variety of aromatic compounds. Main constituents of the essential oils of some important *Cymbopogon* spp. are geraniol. citral, citronellal, citronellol and piperitone. These essential oils also contain a large number of minor constituents. The oil of *C. martinii*, i.e. palmarosa oil is used as a source of geranium odour as true geraniol oil is more expensive. The alcoholic geranium rich in rosaceous

aroma is the principal constituent of palmarosa oil. In India, palmarosa oil is extensively used for adulterating scents (Attar) of roses. Large quantity of oil is exported for use in perfumery. Besides its perfumary value, palmarosa oil has antiseptic properties and some medicinal importance and used in skin diseases, stiff joints etc. The ginger-grass oil obtained from sofia variety is chiefly used as soap perfume.

Cymbopogon flexuosus yields East Indian lemon-grass oil with high citral content. This oil, owing to its high citral content and solubility in 70% alcohol finds acceptance in the world market and preferred over that produced elsewhere. The oil is used specially for the extraction of citral, a starting material for the preparation of ionones (α-ionone and β-ionone). The α-ionone is used in flavours, cosmetics and perfumes, whereas β-ionone is used in manufacturing of synthetic vitamin A (Guenther, 1950). The oil is also used in medicine and as insect repellent.

Cymbopogon citratus(West Indian lemon-grass) is also one of the best source of citral and citronellal. Geraniol and myrcene are the other constituents of this oil. The oil has a lower solubility in 70% alcohol and eventually a lesser economic importance in perfumery trade in comparison to East Indian lemon-grass. Both these oils are equally good for citral preparation which is required in manufacturing of ionones. Jammu lemon-grass oil from *C. pendulus* has high citral content (75-80%) and high yield potential. Thus it can be used both in perfumery and pharmaceutical industries. The oil has same uses like that of *C. flexuosus* oil, and can be used as a supplement to *C.*

flexuosus.

Cymbopogon winterianus yields Java citronella oil and geraniol, citronellal, citronellol and hydroxy citronellal which are commercially important constituents of citronella oil. This oil is used in perfumery, soap, cosmetic and pharmaceutical industries. The oil has been recognized as mosquito repellent and can be used as potential germicide (Dayal and Purohit, 1971). Ceylon citronella oil of *C. nardus* is rich in geraniol (30-40%) but poor in citronellal as compared to *C. winterianus* oil. The oil is chiefly used in cosmetic and soap perfumes and for scenting cheap sprays, disinfectants, detergents and polishes (Guenther, 1950). It is also used in pharmaceutical preparations and found to be antiseptic, carminative, stimulant, sedative, diaphoretic and sudorific (Chopra *et al*., 1956; Kokate *et al*.,1971). The oil of *C. jwarancusa* has large amount of piperitone (upto 80%) and an important ingredient of pharmaceutical preparations used in treatment of cough and rheumatic fever. It is also used as blood purifier and in aromatic tonics in dyspepsea. The oil also has powerful germicidal properties (Dayal and Purohit, 1971).*Cymbopogon coloratus* oil has 34-45% citronellal content. Its odour is reminiscent of both citronella and lemon-grass and thus may be used in perfuming soaps. It is a good supplement to Java citronella, because of its high citronellal contents.

The odour and market-value of *C. caesius* oil are much similar to that of ginger-grass oil since both have geraniol as the chief constituent. The oil of *C. khasianus* has high geraniol content and thus is a good supplement to

palmarosa.

Apart from the economic importance of essential oils of *Cymbopogon* spp., their lignocellulosic wastes also have some potential uses. The spent grass (residues left after distillation) can be used for manufacturing of paper and cardboard as pulping of such materials is easier. This can also be converted into strawboard and fibreboard. Spent citronella grass can also be used in combination with other organic manures. The spent lemon-grass has also been proved to be a good cattle feed.

The *Cymbopogon* industry in India has vast and expanding business potentials due to the wide domestic use of oil and spent grass and the increasing export possibilities of the former. Thus extensive cultivation of different *Cymbopogon* spp. across the country would help to meet the growing demand of these essential oils. It would ensure not only the supply of raw materials but also the in-flow of foreign exchange. As a result of intensive research and development, several high yielding varieties have recently been developed in India.

Mycorrhizae are mutualistic associations between plant roots and fungi which occur naturally throughout the terrestrial ecosystem. Among various forms of mycorrhizae, vesicular-arbuscular mycorrhizae (VAM) are the commonest and widespread, occurring in association with a large number of plant species. There are relatively few absolutely non-mycorrhizal families. The VA mycorrhizal plant species include the most important agricultural

crops and trees such as cereals, grasses, legumes, citrus, coffee, cotton, rubber, tea etc. Vesicular-arbuscular mycorrhizae are known to be present in all types of soil and climate. There are several drought-enduring and drought-resistant VA mycorrhizal plants (Kiran Bala *et al.*, 1989). VA mycorrhizal associations have been reported in hydrophytes as well (Sondergaard and Laegard, 1977; Bagyaraj *et al.*, 1979). The VAM associations have also been reported with salt-marsh plants (Sengupta and Chaudhari, 1990) and those growing in highly alkaline usar land soil (Janardhanan *et al.*, 1994). There are numerous reports of VAM association in mesophytic plants including herbs, shrubs and trees.

The VA mycorrhizae are characterized by the formation of dichotomously branched, haustoria like structures called arbuscules in the cells of root cortex and some of them also produce terminal and intercalary swelling which may be intercellular or intracellular. The arbuscules are dichotomously branched and remain enclosed by the host plasmalemma. These arbuscules are considered as the sites of nutrient exchange between fungus and host-plants. The vesicles are thin walled, multinucleate, expanded, oil rich structures. These are not limited by a septum. Besides the intramatrical phase, VAM fungi also proliferate into the rhizosphere, i.e., extramatrical phase. In extramatrical phase, they produce asexual spores, which may be chlamydospores or azygospores, either free or borne in sporocarp.

The extramatrical hyphae of mycorrhizal fungi grow out from the infected roots into the soil and act as an extension of the plant root system.

Thus the volume of permeated soil becomes much greater with the hyphae of a mycorrhizal fungus than with plant root hairs It improves the uptake of nutrients, specially relatively immobile mineral elements such as P, Zn, and Cu and, to a limited extent, also the ions of Ca. K. Fe. Mg. Mn. Cl, Br and N (Tinker, 1984) resulting in enhanced plant growth. Mycorrhizal fungi also enhance water uptake (Safir *et al.*, 1971) and thus help the plant to withstand the water stress conditions(Parke *et al.*,1983; Safir and Nelsen, 1985). Mycorrhization can ameliorate also the plant response to soil stresses such as high salt levels, different types of toxicities due to mine spoils, heavy metals, or micro-element imbalance. VAM associations decrease the transplant injury (Menge *et al.*, 1978). It promotes the establishment of plants in wastelands (Marx and Altman, 1979), help the plant to withstand high temperatures and impart resistance to certain plant pathogens (Dehne, 1982). The mycorrhizal fungi also alter the soil texture by increasing the extent of aggregation of soil particles and stability (Sutton and Sheppard. 1976). Hence because of these beneficial attributes the VAM fungi have been shown to affect the plant growth in a significant and positive manner (Gerdemann, 1968; Mosse, 1973; Tinker, 1975). The mycorrhizal plants in their natural environment are healthier and grow more luxuriantly than non-mycorrhizal ones. They can also grow better in infertile soil due to their ability to explore greater volume of soil which increases uptake of unavailable nutrients as P,Zn and Cu. In exchange of all these benefits to the mycorrhizal plants, the fungal symbionts obtain carbon from the host plants (Snellgrove *et al.*. 1982; Paul *et al.*, 1985) in the form of carbohydrates produced by photosynthesis.

In view of the importance of *Cymbopogon* spp. in production of essential oils of commerce and absence of study on the impact of VAM association of *Cymbopogon* spp. on growth and productivity and productions of essential oils and its constituents, the present study was undertaken. The major objectives of this study have been to understand symbiotic association system of VAM fungi and *Cymbopogon* spp. and its impacts so that it can be artificially manipulated in order to enhance the essential oil production by the plants for greater commercial benefits.

The objectives of the study have been achieved by studying the following aspects.

(1)Screening of aromatic *Cymbopogon* spp. for vesicular-arbuscular mycorrhizal associations to work out the degree of symbiosis.

(2)Identification of VAM fungi associated with these plants to work out the species of VAM more beneficially associated.

(3)Assessment of seasonal variation of VAM colonization to find out the period of maximum VAM colonization.

(4)Evaluation of the impact on growth and productivity of *Cymbopogon* spp. inoculated with VAM fungi.

(5)Determination of the influences of VAM colonization on nutrient uptake.

(6)The effect of VA mycorrhization of *Cymbopogon* spp. on the production of essential oil and its constituents.

LITERATURE REVIEW

The name mycorrhiza was coined by Frank in 1885. The term mycorrhiza, literally meaning the association of fungi with roots, is derived from two words, mycos and rhiza, i.e: fungus and root. Mycorrhizae are classified into different forms such as ectomycorrhizae, vesicular-arbuscular mycorrhizae, ericoid, arbutoid, monotropoid and orchid mycorrhizae (Harley and Smith,1983). Among these, vesicular-arbuscular mycorrhizae (VAM)are the commonest and wide spread occurring in a large number of species of bryophytes, pteridophytes, gymnosperms and angiosperms. VAM fungi are also the oldest established mycorrhizae as they have been detected some of the oldest land plants such as *Rhynia* and *Asteroxylon* (Kidston and Lang, 1921).

Vesicular-arbuscular mycorrhizae were first recognized and described in the last decade of the 19th century, but the interest in this symbiosis was aroused only in recent years after the establishment of the beneficial attributes of this relationship. VA mycorrhizal associations occur throughout the terrestrial ecosystem. They occur in almost all herbaceous and woody plants (Gerdemann, 1968). Trappe and Fogel (1977) observed VA mycorrhiza in almost 95% of world's known plant species. According to Kendrick and Berch (1985) about 90% of vascular plants normally establish symbiotic relationship with VAM fungi.

VA mycorrhizae are ubiquitous and occur in the plants of arctic, temperate and tropical regions (Hayman, 1978). Most of the plants of arid and

semi-arid range lands of world show VA mycorrhizal association (Trappe, 1981; Miller, 1987). The VA mycorrhizal associations are found to occur with most of the plants of all three main tropical regions of the world, i.e., Asia, Africa and neotropics (Janos, 1983, 1987). However, plants of disturbed sites are often found to be non-mycorrhizal due to reduction in number of mycorrhizal propagules (Reeves *et al.*, 1979). In a recent study, the VAM fungi were not found to have any important role in enhancing growth of poineer species. It had no role in the beginning of successional process in a barren area owing to implaced nutrient poor surface (Titus and Moral, 1998).

In angiosperms, VA mycorrhizae are widespread and found associated with most of the plants except plants of those families which are predominantly ectomycorrhizal. Harley and Harley (1987) made a checklist of mycorrhizae in British flora, in which predominance of VA mycorrhizae can be seen among all species of plants. According to Kendrick and Berch (1985) the most logical way of discussing their host range is to list the groups of plants that do not normally have VA mycorrhiza.

The taxonomy of the VA mycorrhizal fungi is still in a state of active ferment. The classification of VAM fungi in modern terms was done for the first time by Gerdemann and Trappe (1974). They described thirty species of VAM fungi belonging to seven genera in the family Endogonaceae: *Acaulospora*, *Endogone*, *Gigaspora*, *Glaziella*, *Glomus*, *Modicella* and *Sclerocystis*. Ames and Schneider (1979) added a genus *Entrophospora*. and in 1986 another genus *Scutellospora* was added to the Endogonaceae by

Walker and Sanders (1986). Meanwhile two genera, *Glaziella* and *Modicella* were transferred out of Endogonaceae. *Glaziella* was transferred to Ascomycetes by Gibson *et al.* (1986) and *Modicella* to Mortierellaceae (Zygomycetes) by Trappe and Schenck (1982).

Morton (1988) recognized six genera of VAM fungi. Morton and Benny (1990) in their revised classification proposed a new order : Glomales which included all those soil fungi that formed arbuscules. This order is recognized to have three families; Acaulosporaceae, Glomaceace and Gigasporaceae. The six genera of VAM fungi are *Aculospora*, *Entrophospora* (Acaulosporaceae), *Glomus*, *Sclerocystis* (Glomaceae), *Gigaspora*, and *Scutellospora* (Gigasporaceae).

All VAM fungi belonging to these six genera produce two phase mycelial structures (Nicolson, 1967), an external mycelium in the soil and an internal mycelium within the cortex of a mycorrhizal root. The mycelial system produces various characteristic structures in the soil and host and forms the basis of identification. The external mycelium is dimorphic and consist of : thick walled aseptate, coarse hyphae which comprise major portion of the mycelial phase; and thin walled, fine, highly branched, lateral hyphae, become septate at maturity (Mosse. 1959; Nicolson, 1959). The external mycelium produces different kinds of structures in the soil: chlamydospores either singly or in sporocarps (eg. in *Glomus* and *Sclerocystis*), azygospores and soil-borne vesicles (as in *Gigaspora*) as noted by Gerdemann and Trappe (1974), Hall and Fish (1979), and Nicolson and Schenck (1979).

The hypha germinating from the sporocarps, chlamydospores, azygospores becomes the source of infection of host roots. The hyphae penetrate root epidermis, and grow intercellularly and intracellularly through the cortex, extending the infection longitudinally in the root and also penetrating the inner cortex. Descriptions and illustrations on this internal mycelium were made by Janos (1987). In characteristic features of VAM infection, arbuscules are formed in the inner cortex as lateral branches on intercellular hyphae. This dichotomously branched arbuscule is the functional unit of mycorrhiza. Except the species of *Gigaspora*, all VAM fungi produce vesicles on intercellular or intracellular hyphae. The vesicles develop later than arbuscules and considered as storage organs as they contain large number of lipid droplets.

The uninfected portions of the root system get their initial infection from spores/sporocarps in soil or from extramatrical hyphae of nearby mycorrhizal root. Nasim and Iqbal (1992) reported that almost all VAM fungi form propagules in soil under favorable conditions. The propagules germinate and establish contact with host plants and cause infection. In most of the studies on infection process, spores or infected root segments are used as inoculum. Investigations have shown that spores of VAM fungi could germinate on agar medium as well as slides buried in soil (Mosse and Phillips, 1971, Mosse and Hepper, 1975; Powel, 1976; Hepper, 1981). Hepper and Mosse (1975) found that percentage spore germination on slides buried in unsterile soil was similar to that of spores germinated axenically on water agar.

When spores are source of infection, germination followed by the growth of one or more germ tubes results in simple mycelium of few centimeters. If susceptible roots are present, the increased growth is observed (Harley and Smith; 1983). Mosse and Hepper (1975) and Powell (1976) showed that inspite of this increased mycelial growth in the presence of roots, hyphae may not appear to make directional growth towards roots if they are not close to them. But Koske (1982) observed a reaction of germ tubes which were present at a distance of 11mm from roots.

After spore germination the main hyphae of about 20-30 μm diam. give rise to fan shaped complex of septate lateral branches of 2-7 μm diam. and these lateral, narrow hyphae cause infection on the root. The development of these 'pre-infection fans' vary with the source of inoculum. If the hyphae are from germinated spore, they show directional growth and fan production of greater extent, whereas if the infection occurs from infected root segment, the pre-infection branching is limited or even absent. Other than spores, infection can also take place from hyphae growing out of living infected roots; hyphae freshly detached from roots (Johnson, 1977; Hepper 1981); infected roots severed from their parent plant as they have attached spores and hyphae; and vegetative hyphae present in dead or senescent roots (Dowding 1959; Tommerup and Abbott, 1981) Even resting structures, i.e., vesicles may also persist in dead roots and later germinate like external spores.

The earliest studies by Nicolson (1959) on the infection process in roots of grass showed that hyphae finally make contact with epidermal cells

or root hairs, and produce appressoria from which infection pegs arise followed by the penetration of epidermis or root hair cells. Gianinazzi (1991) suggested that formation of this more or less well defined appressoria indicates occurrence of some kind of recognition phenomenon at an early stage of VAM infection.

Formation of appressorium on the root epidermis is rapidly followed by the penetration of the epidermal and cortical cells by hyphae. Some workers (Gianinazzi-Pearson *et al.*,1981; Jacquelinet-Jeanmougin *et al.*, 1988; Kinden and Brown, 1975; Mosse, 1962; Kaspari, 1975) suggested that enzymes are presumably involved in the penetration process. Holley and Peterson (1979) described a peg like projection of the hyphae which caused invagination of host cell wall and also of plasmalemma. Harley and Smith (1983) observed penetration of tissues by VAM fungi and concluded that penetration does not seem to depend on active hydrolytic enzymes produced by fungal hyphae.

Having entered the root, the fungal hyphae start spreading and branching intercellularly and intracellularly in the cortical region. The fungal spread within the outer cortex is restricted to some extent to facilitate the growth and differentiation of fungal hyphae in the middle cortex. In the outermost cortical cells, the fungus may form hyphal coils or it turnover the deeper layer of cells. In the inner cortex of fungal hyphae differentiated to produce its characteristic structures : arbuscules and vesicles.

The arbuscules are surrounded by host plasmalemma and found on specialized branches of fungal hyphae which enter the cortical cells. The arbuscule differentiation may be related to modifications in fungal wall metabolism, which are controlled by host plant (Bonfante-Fasolo, 1988). Arbuscule formation does not occur in non-host plants where infection is restricted to intercellular hyphae (Glenn *et al.*, 1985 Gianinazzi-Pearson and Gianinazzi, 1988). Gianinazzi-Pearson and Gianinazzi, (1988) confirmed importance of host plant for arbuscule development. The vesicles develop later than arbuscules in the process of infection in or between the root cells. These are terminal swellings on intercellular or intracellular hyphae in the middle or outer cortex. Vesicles are thin walled expanded structures, not delimited by a septum and contain large amount of lipids.

Vesicles are certainly storage structures and may be involved in temporary storage of modified photosynthates received from the host plant. But these organs are not formed by the species of *Gigaspora* and due to this, Daft and Nicolson (1974), Morton (1990 a, 1990 b) referred the VAM as **arbuscular mycorrhiza** and consequently VAM fungi are also called **AM fungi.** Despite of all these hyphal modifications inside the root, i.e., the intramatrical phase, fungus also has an extramatrical phase. The extramatrical hyphae grow extensively along the root surface and initiate secondary infection units after the establishment of primary infection. They also grow in the surrounding soil, form a network of hyphae and act as an extention of the plant's root system. A hyphal network associate with a living plant is capable of infecting others

growing in its vicinity (Read *et al.* 1976; Chiarello *et al.* 1982; Francis and Read 1984; Francis *et al.*, 1986). Evans and Miller (1990) further suggested that its infectivity might persist for extended periods in the absence of a living symbiont.

The extramatrical hyphae produce large, rounded presumably asexual spores on their terminal branches. The extramatrical spores are found singly or in aggregation upto 1cm diam., i.e., sporocarps. Rarely, they are found in roots and sometimes in relatively protected spaces. These spores have variable size, colour, shapes and wall structures and filled with storage lipids.

The network of extramatrical hyphae ramifies in the soil and explore larger volumes of soil than do non-mycorrhizal plants. Rhodes and Gerdemann (1975) observed extension of these hyphae upto 8 cm from the root and suggested their active participation in translocation of mineral to the host. Many other workers (Hattingh *et al.*, 1973; Gianinazzi-Pearson and Tinker, 1975; Cooper and Tinker, 1981) also had successfully demonstrated their involvement in translocation of minerals. Out of these, uptake of soil phosphorus is mainly affected which is improved by the extramatrical hyphae as they increase the root of absorptive surface area (Hayman, 1983). Safir *et al.* (1971) suggested that extramatrical hyphae may perform similar function in water uptake. Hardie (1985) also tried to explain the importance of extramatrical hyphae in water uptake by his experiment on clover and leek with *Glomus mosseae*.

Vesicular-arbuscular mycorrhizal fungi play a vital role in the mineral

nutrition of a host plant by their extensive hyphal ramification in the soil (Trappe, 1981). Now it has been established that VA mycorrhiza improve the uptake of relatively immobile mineral elements such as phosphorus, zinc and copper and also to a limited extent, ions of Ca, K, Fe, Mg, Mn, Cl, Br and N resulting in enhanced plant growth. (Gerdemann, 1968; Mosse, 1973; Tinker, 1984). Mosse (1957) was the first to have clearly demonstrated the increased amounts of potassium, iron and copper per unit weight of tissue in mycorrhizal plants. Gerdemann (1964), Daft and Nicolson (1966) and Baylis (1967) established that tissue concentrations of phosphate were higher in mycorrhizal plants. VAM fungi are proved beneficial in infertile soils or where such elements are less available or deficient, by increasing the efficiency of mineral uptake.

There are many evidences suggestive of increased efficeincy of mycorrhizal plants in phosphorus uptake (Sanders and Tinker, 1973; Hattingh *et al.*, 1973; Cooper and Tinker, 1978; Gnekow and Marschner, 1989; Jungk and Classen, 1989). Absorption of phosphate by mycorrhizal fungi is followed by the synthesis of inorganic polyphosphate in the fungal vacuoles in the external hyphae (Callow *et al.*, 1978) and passed to the arbuscules for transfer to the host (White and Brown, 1979). This flow of phosphorus occurs in the presence of the acid phosphatases (Gianinazzi *et al.*, 1979) during the arbuscule life span (Cox and Tinker, 1976) or senescence (Kinden and Brown, 1976).

VAM fungi have been found very effective and able to utilize extremely small quantities of fertilizer. These days the use of phosphatic fertilizers has

increased to a great extent. These fertilizers are costly and associated with pollution problems and so their use especially in developing countries is losing its due relevance. Due to their ability to improve the fertilizer efficiency, VAM fungi are being regarded as biofertilizers and their use in agriculture is being encouraged. It is also important to know which plant species derive more benefit from mycorrhiza (Pope *et al.*, 1983). Some plant species are highly mycorrhiza dependent, while others are less dependent.

In additon to their role in phosphate assimilation, VAM fungi have also been reported to play an important role in nitrogen transfer to and from plants (Raven *et al.*, 1978). There is no evidence that mycorrizal fungi or any othert fungi can fix atmospheric nitrogen. In mycorrhizal plants where increased concentrations of nitrogen have been recorded, they must result from increased uptake from the soil. Raven *et al.* (1978) reported that VAM fungi assimilate and transport both, ammonium ions and some organic nitrogen compounds to their host plants. Direct transfer of N via hyphal connections between roots of closely asociated plants was suggested and demonstrated in several experiments (Ames *et al.*, 1983; Van Kessel *et al.*, 1985; Francis *et al.*, 1986; Haystead *et al.*, 1988).

Increased uptake of phosphorus and nitrogen are not the only effects of VAM fungi on plant growth. They also stimulate the uptake of zinc, copper, sulphur, potassium and calcium but not as markedly as phosphorus (Cooper and Tinker, 1978). It is now established that mycorrhizal infection could alter the zinc and copper uptake (Tinker, 1978; Gildon and Tinker, 1983) of plants.

The mechanism is not known in detail, but VAM hyphae have been shown to translocate zinc. Also, there are reports of translocation of sulphur, calcium (Rhodes and Gerdemann, 1975, 1978 a, 1978 b) and potassium (Possingham and Groot-Obbink, 1971). But their translocation appears to be less efficient than translocation of phosphorus.

VAM also decrease the acquisition of certain elements by the plants when they are in excess, nearly at toxic level in the soil as reported in the case with heavy metals in polluted environments (Mosse, 1986) or with Mn in acid soils (Arines and Vilarino, 1989; Arines *et al.*, 1989). Reduced Mn and Fe uptake has also been reported by Pacovsky, (1986), Pacovsky *et al* (1986 a, 1986 b), Raju *et al.* (1990). Azcon *et al.* (1991) observed the buffering effect of VAM in presence of nutrient (Ca and Mg) excess in a calcareous soil.

VA mycorrhizae also have been shown to increase water uptake, and/or alter the physiology to reduce stress response to drought (Safir *et al.*, 1971; Levy and Krikum, 1980; Safir and Nelsen, 1985). Mosse and Hayman (1971) observed wilting in nonmycorrhizal onion plants when transplanted whereas mycorrhizal plants did not wilt. Levy and Krikum (1980) in their experiment with *Citrus jambhiri* and VAM fungus observed that major effects of mycorrhizal infection was an increase in transpiration influx, stomatal conductance, both during stress and recovery period, and an increased rate of photosynthesis per unit leaf area during recovery period. Hardie and Leyton (1981) and Allen *et al.*, (1981) observed that mycorrhizal plants generated lower leaf water potentials in the case of low soil water potential. Hardie and Leyton

(1981) also observed that mycorrhizal clover plants wilted at lower soil water potentials than non-mycorrhizal, but they recovered faster on rewetting.

The increased phosphorus uptake, improved mineral nutrition and improved soil moisture uptake results in the increased growth of VA mycorrhizal plants. Abbott and Robson (1984), Smith and Gianinazzi-Pearson (1988) pointed out that improved phosphate uptake is the most frequent and primary cause of growth and yield enhancements in VA mycorrhizal plants. Now, it is an accepted fact that mycorrhizal plants in their natural environment are normally healthier and grow more profusely than non-mycorrhizal plants.

The VAM fungi have been established to have significant effect on plant growth (Gerdemann, 1968; Mosse, 1973; Tinker, 1975;). Asai (1944), in his studies on mycorrhizal infection and nodulation of legumes, obtained improved growth of hosts associated with vesicular-arbuscular mycorrhizae. Gerdemann (1964) reported enhanced growth of mycorrhizal plants. Daft and Nicolson (1966) reported that VA mycorrhizal plants have 4.5 times greater dry weight than uninoculated (control) plants. Daft and Nicolson (1966, 1969b, 1972) were among the first to demonstrate that development of mycorrhizal roots and their effect on plants growth is greater in soils with low or imbalanced nutrition. Recent studies (Cooper, 1983; Green *et al.*, 1983; Huang *et al.*, 1983; Habte and Aziz, 1985; Chen, 1985; Ramaraj and Shanmugum, 1986; Kandasamy *et al.*, 1986; Senapati *et al.*, 1987; Khan *et al.*, 1988;) now confirm beneficial role of VAM fungi in increasing plant growth. Chen (1985) observed manifold increase in biomass of *Aleurites montana* on inoculating

its seedlings with VAM fungi. Ramaraj and Shanmugam (1986) also obtained increased growth of some pulses by soil or seed inoculation with mycorrhizal fungi. Khan *et al.* (1988) reported that VA mycorrhizal infection greatly improved the growth and nutrient uptake of rice plants. Growth and development of seedlings were also found to be affected by the presence or absence of VA mycorrhizal fungi (Kandasamy *et al.*, 1986). A noticeable increase in the seedling height, numbers of branches per seedling, roots per seedling, root length and dry weight of seedlings of *Pyrethrum* spp. occurred due to VAM inoculation and inoculated plants transplanted in the field showed an advancement of flowering (Kandasamy *et al.*, 1986). The improvement in seedling growth due to VAM inoculation is reported in a number of plants (Palipane and Bandra, 1985; Melichar *et al.*, 1986 and Dixon, 1988). The increase in growth of VA mycorrhizal plants is attributed mainly to improved moisture uptake by the host (Ross and Harper, 1970; Mosse,1973; Fitter, 1977; Allen and Allen, 1984).

VA mycorrhizal fungi have also been found to reduce the effects of several pathogens on their hosts. Two main groups of soil-borne pathogens have been studied : fungi and nematodes. In the presence of VAM, reduction in pathogen population or in the severity of disease in the host plant has been demonstrated for both groups. According to Harley and Smith (1983) interactions between VAM mycorrhizal infections and plant disease are complex and may involve : (a) competition for actual sites of infection in the root; (b) changes in the nutritions of the host plant; and (c) increase of tolerance of the

plant to infection where the mycorrhizal fungi compensate for the damage to the root caused by the pathogen.

Several workers reported the interactions between VAM fungi and soil borne fungal pathogens (Dehne, 1982; Bagyaraj, 1984; Schoenbeck, 1979; Garcia-Garrido and Ocampo, 1987). Perrin (1990) pointed out that the effect of VAM occurs only when the mycorrhizal fungi invade the roots before the pathogens, whereas in those experiments, where the plants were inoculated simultaneously with both the pathogens and VAM fungus, no reduction in disease was seen (Rosendahl, 1985; Bartschi *et al.*, 1981). But VAM fungi and could be adapted in case of transplanted fields and horticultural crops, where plants with pre-established mycorrhizae could be planted (Paulitz and Linderman, 1991).

Plant parasitic nematodes, like VAM fungi, colonize the root cortex and are also present in the rhizosphere. Many workers suggested that mycorrhizal fungi act as biological deterrents to nematode pathogens (Marx, 1972; Schenck and Kellam, 1978; Schoenbeck, 1979; Schenck, 1981; Hussey and Roncadori, 1982). However, in some instances VAM colonization had no effect on nematode population (Grandison and Cooper, 1986; Cason *et al.*, 1983). In other studies the nematode populations were found consistently higher in mycorrhizal plants as reported by Atilano *et al.* (1981). Paulitz and Linderman (1991) suggested that reduction in the symptoms and nematode effects might be dependent on the level and timing of VAM infection. The effect of plant parasitic nematodes on the VAM fungus is variable, i.e., form

decreased spores production (Atilano *et al.*, 1981) to no effect (Kellam and Schenck, 1980; Strobel *et al.*, 1982; and Mac Guidwin *et al.*, 1985) to increased spore production. The response of mycorrhizal infection to the presence of nematodes also ranged from enhancement (Bagyaraj *et al.*, 1979) to no effect (Kellam and Schenck, 1980; Cooper and Grandison, 1986) to inhibition (O'Bannon and Nemce, 1979).

VAM fungi also interact with rhizobacteria as they are also present in the same rhizosphere together with plant pathogenic fungi and nematodes. Meyer and Linderman (1986) demonstrated that bacterial populations differ in VAM and non-VAM plants. Several workers reported interactions of VAM fungi with symbiotic nitrogen fixers (Bethlenfalvay and Yoder, 1981; Tilak, 1985; Bayne and Bethlenfalvay, 1987), free living nitrogen fixers (Barea *et al.*, 1983; Pacovsky *et al.*, 1985; Subba Roa *et al.*, 1985; Tilak and Singh, 1988), plant growth promoting bacteria (Prikryl and Vancura, 1980; Azcon-Aguilar *et al.*, 1981) and also with biological control agents (Krishna *et al.*, 1982; Paulitz and Linderman, 1988).

Some *Cymbopogon* spp. have been reported to be associated with a number of VAM fungi (Barthakur and Bordoloi, 1990; Gupta *et al.*, 1990; Janardhanan *et al.*, 1990; Gupta and Janardhanan, 1991). Gupta *et al.* (1990) recorded the association of an unidentified species of *Glomus* with palmarosa (*C. martinii* var. motia). In a later study Gupta and Janardhanan (1991) worked out the association of *G. aggregatum* with *C. martinii*. In a preliminary study, Barthakur and Bordoloi (1990) observed VAM association on a number of

Cymbopogen spp. However, the identity of VAM fungi was not established by these workers. There are only few reports on seasonal variations of VAM fungi (Lopez-Sanchez and Honrubia, 1992; Vardavakis, 1992; Shamim *et al.*, 1994).

Vesicular-arbuscular mycorrhizal (VAM) fungi are present in nearly all soils and colonise roots of the great majority of plants. This symbiotic association is proved very beneficial to host plants. Although essential oil producing plants are cultivated throughout the world, India accounts for 1.6% of the global trade of essential oils and aromatic compounds of plant origin. International trade involves about 62 types of essential oils. However, India's export is limited to a few of them (Anonymous, 1997). The lemon-grass (*Cymbopogon flexuosus*) is mainly grown in Kerala state on an area of about 30,000 ha and major part of the produce worth Rs. 10 million (US $ 0.27 million approx.) is exported annually (Anonymous, 1997). The grass yield ranges from 18 to 25 t ha^{-1} and oil yield between 60 to 72 kg ha^{-1} (Anonymous, 1997). The trade statistics, however, revealed that the India's export of lemon-grass oil registered a steep fall from 259 t in 1985 to 65 t in 1995 (Anonymous, 1985 to 1996).

The tall perennial palmarosa grass (*Cymbopogon martinii*) grows wild in dry scrub forests in the states of Andhra Pradesh, Madhya Pradesh, Maharashtra and Karnataka. The commercial cultivation is coming up. The oil yield varies from 10 to 100 kg ha^{-1} yr^{-1} (Anonymous, 1997). The bulk of oil distilled from the grass is exported. The trade statistics revealed that about

2.58 t of palmarosa oil worth Rs. 0.96 million was exported in 1991and 2.16 t was imported in 1995. The export increased to the order of 5.4 t (worth Rs 3.51 million) in 1995 and the import decline to about 0.4 t yr^{-1} (Anonymous, 1985 to 1996).

The stemless perennial citronella-grass (*Cymbopogon winterianus*), recently introduced in India, is being cultivated on about 2000 ha in states of Assam, Gujarat and Karnataka. The fresh herbage yield varies from 16 to 20 t ha^{-1} and the oil yield between 100 to 150 kg ha^{-1} (Anonymous, 1997). A large quantity of this oil is imported (Anonymous, 1997). The trade statistic revealed that in past 12 years the export of citornella oil began with 0.11 t in 1989 which increased to the tune of 29.5 MT in 1995 and 77.2 MT in 1996 (Anonymous, 1985 to 1996). However, nearly 111.2 MT of oil worth Rs 67.6 million (US $ 2 million approx.) was imported in 1991. On introduction of this crop in India, the oil import fell down to the order of 1.2 t worth Rs 0.161 million (US $ 4600 only) in 1991. The quantity of oil imported further declined to 0.32 MT (worth Rs. 0.18 m, US$ 5000 in 1996 (Anonymous, 1985 to 1996).

The attempts made to determine the impact of mycorrhizal association on the essential oil content of such plants are limited to fewer reports (Sirohi and Singh, 1983; Abdul-Khaliq, 1993). In this experiment, studies were made to find out the impact of VAM association on the quality and quantity of essential oils of *Cymbopogon* spp.

The perusal of the available literature shows that there has been only a

meagre investigation on the mycorrhizal associations of *Cymbopogon* spp. The available information is inadequate and further investigations would be interesting and rewarding.

MATERIALS AND METHODS

Selection of plants

The five *Cymbopogon* spp. selected for VAM screening are listed below.

1) *C.caesius* (Nees) Stapf. (Kachi-grass)

2) *C.flexuosus* (Steud.) Wats. (East Indian Lemon-grass)

3) *Cymbopogon martinii* (Roxb.)Wats. (Palmarosa)

4) *C.pendulus* Stapf. (Jammu Lemon-grass)

5) *C.winterianus* Jowitt. (Citronella)

Location of experimental site

The five species of *Cymbopogon* selected for the field study are cultivated and maintained at the experimental farm of the Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow, for experimental purposes. The field and glass-house experiments of the present study were conducted at CIMAP, Lucknow, India.

Collection of soil and root samples

The upper 25 cm layer of the soil around each plant was examined for VAM propagules during the investigation. Nearly 1 to 1.5 cm thin upper surface of soil was scrapped and removed before sampling . The rhizosphere

soil was collected carefully along with fine roots having their cortical portions intact. Five rhizosphere samples of each selected *Cymbopogon* sp. were collected randomly. The fine roots were sorted out, gently washed with water and processed fresh for assesment of VAM colonization. The residual rhizosphere soil was used for the isolation of VAM spore. To find out the extent of seasonal variation in VAM association, the root and soil samples were collected at one month periodic intervals throughout the year.

Isolation of VAM spores from soil

The VAM spores were isolated from soil samples following wet sieving and decanting method of Gerdemann and Nicolson (1963). The isolation technique followed in the study is described stepwise as follows :

Step I : After separating fine roots from soil samples, the residual rhizosphere soil sample was mixed thoroughly.

Step II: A suspension of 100 g of residual soil sample was made in a 2000 ml beaker by adding water and stirring it thoroughly.

Step III: The suspension was kept still for few minutes to allow the heavier particles to settle down. The settled residue was collected and supernatant was processed as in step IV.

Step IV: The supernatant muddy suspension was poured through coarse sieve (610 mm) for the removal of large soil particles and organic debris. The suspension was then passed through another sieve fine enough to retain

smaller spores.

Step V : The residue collected (as in step III) was resuspended in water and process of the step IV was repeated.

Step VI: The material retained in the fine sieve during step IV and V was thoroughly washed with water. The volume was made to 50 ml with water and transferred to a 100 ml beaker and hitherto referred to as spore suspension.

Quantitative analysis of VAM spores

The spore suspension was stirred to make the homogenous distribution of spores. From the homogenous spore suspension, one ml was pipetted into nematode counting slide (Hawksley, UK) for counting the VAM spores under stereoscopic microscope. Ten counts were made from each spore suspension and the average of these counts was calculated to determine the density of spores per unit volume. Eventually the spore count per unit volume of suspension was converted to determine number of spores per gram dry soil.

Hundred gram soil of each sample was dried in oven at 80-100^{0}C for 12-15 hours. The dried samples were cooled and weighed in order to find out the moisture content.

Processing of root samples and assessment of VAM colonization

The Phillips and Hayman's technique (1970) of clearing and staining the roots was followed for studying the root samples. The technique involved

(i) thorough washing of roots with tap water and chopping them into 0.5- 1 cm segments, (ii) clearing of root segments in 10% KOH at 90°C for 30 min. and rinsing with tap water (iii) treatment of KOH cleaned roots with 1N HCl for 8-10 min. and rinsing with distilled water, (iv) staining with 0.05% trypan blue prepared in lactophenol (lactic acid: glycerine: DW: phenol :: 1:2:2:1) The stained root segments were picked and mounted over slide in lacto-glycerine.

The assessment of VAM colonization in the roots was made as done by Abdul-Khaliq (1993) based on the method described by Beirmann and Linderman (1982). The various steps taken were : (i) twenty five to fifty stained roots segments of 0.5 to 1.0 cm length were mounted on the slide, (ii) the mounted root segments were observed under a compound microscope (at 10X x 10X) fitted with an ocular micrometer scale (having 100 divisions), (iii) the portion of root pieces with vesicles, arbuscules and hyphae of the fungus covering 1 division of ocular scale was presumed to be as 1% infection. The data so recorded was treated as frequency distributions of VAM fungi on the root sample, hitherto referred to as percentage of root colonization by VAM fungi.

Identification of VAM fungi

A drop of the spore suspension obtained after sieving was observed under a stereoscopic microscope. The spores were picked up with the help of fine wooden pick and transferred to lactophenol on a slide. At least 25-50 spores with identical morphology were picked, mounted and observed under

a compound microscope. The spore colour, shape, size, wall thickness, number of walls, length and width of subtending hyphae, pattern of attachment of subtending hyphae to the spores etc. were recorded separately for each spore under observation. The dimensions were measured by ocular micrometer and values were converted to microns. The morphological characters of the spores were compared with the key (Hall and Fish, 1971) and finally with the 'Manual for the Identification of VA Mycorrhizal Fungi' (Schenck and Perez, 1988). Some spores from the sample of each plant were mounted and maintained as permanent specimens.

Selection of test materials

Three *Cymbopogon* spp., viz; *C.martinii* (Roxb.) Wats. (Palmarosa), *C.flexuosus* (Steud.) Wats. (East Indian lemon-grass), and *C.winterianus* Jowitt (citronella) were selected as test plants for further studies. The selected species have greatest economic value due to their commercially important essential oil. For inoculating the test plants, *Glomus aggregatum* Schenck and Smith emend Koske, *G. fasciculatum* (Thaxter) Gerdemann and Trappe emend Walker and Koske, and *G. mosseae* (Nicol. and Gerd.) Gerdemann and Trappe were selected as test VAM fungi.

Maintenance of host plants and monospore culture

For developing monospore culture (inoculum) of *Glomus aggregatum*, *G. fasciculatum* and *G. mosseae* the host plants were maintained as follows:

Two plants, palmarosa (*Cymbopogon martinii* var. motia) and Rhodes grass (*Chloris gyana*) were used for developing monospore culture of the VAM fungi because of their susceptibility to VAM infection and multiplication of the VAM fungi as observed in preliminary studies. The seeds of these plants were surface sterilized with 0.01% $HgCl_2$ solution for 90-120 seconds, washed thoroughly three to four times with sterilized distilled water to remove the traces of mercuric chloride. The surface sterilized seeds were sown in 10 cm diam. pots filled with garden soil. The soil filled pots were autoclaved at 121°C for one hour before seed sowing. Irrigation was done with sterilized distilled water. After the germination, one or two seedlings were transferred to each clay pot of 10 cm diam filled with garden soil and autoclaved. These pots were irrigated when required with sterilized distilled water.

Isolation of VAM spores and inoculation

The VAM spores were extracted from the rhizosphere soil by wet sieving and decanting method of Gerdemann and Nicolson (1963) as described previously. The isolated spores were transferred into filter paper cones. The cones were made up by utilizing steam sterilized filter paper circles of approximately 50 mm diameter. A solution of 2% chloramine T and 0.02% streptomycin sulphate was poured drop by drop, over the spores for 20 min. (Hepper and Mosse, 1975) for surface sterilization. The spores were then thoroughly washed by dropping sterilized distilled water into the cones.

For the inoculation of host plants, small amount of soil from the root zone of established seedings was removed and a small pit was made. Then the surface sterilized spores were transferred from the cones to the pits. The pits were covered with soil. The inoculation plants were irrigated with sterilized tap water whenever required.

Multiplication of VAM fungi

From each inoculated pot, rhizosphere soil was collected after five to six weeks for examination of VAM colonization. The plants showing infection on their roots were separated from other pots. The seedlings from these pots along with rhizosphere soil and root system were transferred to sterilized soil contained in 25cm diam pots. These pots were also irrigated with modified Hoagland's medium (in sterilized tap water) without phosphorus supplementation at time interval of 35-40 days to facilitate better growth of the VAM fungus in the pot culture. Normally the pots were irrigated with sterilized tap water, as and when required.

To keep all the monospore cultures pure and to avoid contamination by insects and bacterial phathogens, rodents and also other VAM fungi, all general glasshouse sanitation practices were followed.

Preparation of soil

From the CIMAP experimental farm, garden soil was collected and used for experiment. The collected soil was thoroughly mixed with farm yard

manure (FYM) in the ratio of 3:1. With this soil manure mixture 25 cm diam. clay pots were filled. These pots were then autoclaved at 121°C for two hours.

Plant propagating materials

For the preparation of plant propagatory material, slips of *C. martinii*, *C. flexuosus* and *C. winterianus* were collected from single clones of these plants growing in CIMAP farm. After thoroughly washing all the roots were removed to avoid any infection. Then the lower portions of the slips were surface sterilized by dipping in 50% alcohol for 30 sec. and washed with sterilized water 3-4 times. The slips were again surface sterilized by sodium hypochlorite (1% available chloride).

Preparation of inoculum

The monospore cultures of *G. aggregatum*, *G. fasciculatum* and *G.mosseae* (which were maintained in pots on palmorsa under glass house conditions) were used for inoculation. The soil and VAM colonized roots of the culture pots were thoroughly mixed. The soil containing approx. 200 VAM spores including extramatrical hyphae and infected root segments were used as inoculum for each treatment.

Planting of test plants

Twenty five cm pots filled with garden soil were steam sterilized at 121° for 2 hours. The pots were inoculated with each of the VAM fungus separately as per the schedule given below. For inoculation, a furrow of about

10 cm was made in the soil in the centre of each pot by removing soil. Then the VAM inoculum of required fungus was spread in these furrows. After placing the inoculum, surface sterilized slips of the plant species were planted in the furrows over the inoculum in each pot. The pots were kept in glass house and irrigated with tap water as and when required.

Inoculation schedule

Inoculation of the soil with VAM fungi was done prior to the planting of slips according to the following schedule :

Control

Cymbopogon flexuosus (uninoculated)

C. martinii (uninoculated)

C. winterianus (uninoculated)

Single inoculation

C. flexuosus x *Glomus aggregatum* / *G.fasciculatum* / *G.mosseae*

C. martinii x *G.aggregatum* / *G.fasciculatum* *G.mosseae*

C. winterianus x *G.aggregatum* / *G.fasciculatum* *G.mosseae*

Combined inoculation

C. flexuosus x *G.aggregatum* + *G.fasciculatum* + *G.mosseae*

C. martinii x *G.aggregatum* + *G.fasciculatum* + *G.mosseae*

C. winterianus x *G.aggregatum* + *G.fasciculatum* + *G.mosseae*

Each of the above treatments were replicated five times.

Recording of data

Initial readings of the following parameters were taken after three months of plantations.

1. Plant height
2. Number of tillers
3. Shoot fresh weight
4. VAM colonization (% root infection)
5. VAM population (spores g^{-1} soil)

After recording the data, plants were trimmed at a height of 10-15 cm above the soil level. Final readings were taken after 12 months (at the time of termination of the experiment) when the plants had regrown in full after the clipping.

At the time of termination of the experiment the following parameters were accounted for :

1. Plant height
2. Number of tillers
3. Shoot fresh weight

4. VAM colonization (% of root infection)

5. VAM population in rhizosphere

6. Total oil content

7. Amount of the principal constituents of oil (%)

8. Total N,P,K, Cu and Zn available in soil

9. Total N,P,K, Cu and Zn in plants.

Estimation of essential oil content

Essential oils were extracted from the whole plants by hydrodistillation using ' Clevenger's apparatus'. Samples weighing 100 g of each inoculated and control plants were collected afresh and chopped into pieces and transferred to the flask of apparatus containing water and distilled for 2 hours. The total oil content was calculated on fresh weight basis. The geraniol, citral and citronellal contents, the major constituents of *C. martinii* (palmarosa), *C. flexuosus* (lemon-grass) and *C. winterianus* (citronella) respectively were analysed by gas liquid chromatography (GLC). The GLC analysis was performed by a ' Perkin Elmer Model 13920 B' equipped with thermal conductivity detector (TCD). A gas column 6 ft. x 1.5 inch with 20% reoplox, 400 on chromosab WNAW, was used at 121°C, 150 thermal operations. The percentage geraniol, citral and citronellal contents were determined by Vesta 401 integrater attached with GLC apparatus.

Soil chemical analysis for macronutrients and micronutrients

Soil from each experimental pot was collected after sterilization and before planting and also harvest. The samples were then air dried, powdered and passed through the 2 mm sieve. The chemical analysis of the soil for N,P,K,Cu and Zn were carried out following standard procedures (Jackson, 1973).

Analysis of nitrogen

Soil mineralizable nitrogen was estimated by the alkaline permanganate method as described by Subbiah and Asija (1956). Twenty gram soil was taken in 800 ml Kjeldahl flask to which 20 ml of water + 100 ml of $KMnO_4$ (0.32%) + 100 ml NaOH (2.5%) solutions were added. One ml of liquid paraffin was added in solution to prevent frothing during boiling, and few glass beads were added to prevent bumping. The contents were distilled in Kjeldahl assembly at a steady rate and the liberated ammonia was collected in a 250 ml conical flask containing 20 ml of 2% boric acid solution (with mixed indicators: methyl red and bromocresol green: 20 ml/lt.). The pink colour of the boric acid solution was turned to green with the absorption of ammonia. The 100 ml of distillate was collected for about 30 min. which was then titrated against 0.02 N H_2SO_4 to the original pink shade. A blank was also run for the final calculation. The nitrogen content was calculated by the formula:

Mineralizable nitrogen (ppm) $= R \times 0.02 \times 0.014 \times 10^6$

$= R \times 0.02 \times 0.05 \times 0.014 \times 10^6$

where "R" is the volume of 0.02 NH_2SO_4 required for titration.

Analysis of phosphorus

For the estimation of available phosphorus in soil Olsen's method (Olsen *et al.*, 1954) was followed. The extracting reagent for Olsen's P is 0.5 molar sodium bicarbonate (pH 8.5).

To 2.5g of soil in 100 ml conical flask, a little amount of Darco G60 was added followed by 50 ml of Olsen's reagent in a 100 ml conical flask. A blank was run without soil. The flasks were shaken for 30 min. on shaker and the contents were filtered immediately through filter paper (Whatman No. 1) into clean and dry beakers. From this filterate, phosphorus was estimated colorimetrically by Dickman and Bray's (excess acid) procedure (Dickman and Bray, 1940).

Dickman and Bray's reagent was prepared as : 15 g of ammonium molybdate was dissolved in 300 ml of warm water (about 60^0C), cooled and filtered. To this, 400 ml of 10 N HCl was added and volume was made upto one litre.

Five ml of soil extract (filtrate obtained from shaking the soil in Olsen's reagent) was pipetted into a 25 ml volumetric flask. To this Dickman and Bray's reagent was added drop by drop with constant shaking till the effervescence due to CO_2 evolution ceased. The neck of the flask was washed down with distilled water and the volume was made approximately 22 ml. Then

one ml of the diluted stannous chloride solution (from 40% $SnCl_2$ stock solution) was added and volume was made upto 25 ml. The intensity of blue colour was measured at 660 nm using a spectrophotometer just after 10 minutes. The concentration of P was determined from the standard curve. Available phosphorus content was calculated as follows :

Available P (μg.) = R x [50/2.5] x [25/5] = R x 100

where, R = μg P in the aliquot (obtained from the standard curve).

Analysis of potassium

Available potassium (exchangeable and water soluble) was determined from neutral normal ammonium acetate extract of soil (Jackson, 1973). For this 5 g soil was shaken with 25 ml neutral normal ammonium acetate (2N acetic acid, glacial + 2N ammonium hydoroxide in 1:1, pH7) for 5-10 min. and filtered immediately. Potassium concentration in extract was determined by flame photometer.

Analysis of micronutrients (Cu and Zn)

Micronutrients (Cu and Zn) of soil were determined by the method of Lindsey and Norwell (1969). Micronutrients were extracted from the soil in DTPA (Diethylene triamine penta acitec acid) reagent. The DTPA was prepared by dissolution of 1.967g DTPA + 1.470g $CaCl_2.2H_2O$ in 20-25 ml of double distilled water and 13.3 ml of 0.1 M triethanolamine (TEA) was added to it. Then the volume was made 1000 ml and pH was adjusted at 7.3.

Ten gram of soil was shaken in 20 ml of DTPA reagent for two hours in a conical flask. The extract was filtered through Whatman filter paper (no.42). Micronutrients in the soil were estimated by atomic absorption spectrophotometer.

Plant chemical analysis for macronutrients and micronutrients

Samples of plant materials were dried in oven at 60^0 for 24 h. Well dried samples were finely ground and passed through 2 mm sieve.

Estimation of NPK

The analysis was done following the official methods of analysis (1995).

Digestion : 100 mg of the dried powder of each sample was transferred to a 50 ml flask to which 2 ml sulphuric acid was added. The contents of the flasks were heated on temperature controlled hot plate for about 2 h. The contents of the flasks were turned black. After cooling the flask for 15 min. 0.5 ml 30% hydrogen peroxide was added drop by drop and the solution was heated again till the colour of solution changed from black to light yellow. After cooling for about 30 min. an additional 3-4 drops of 30% hydrogen peroxide was added, followed by heating for another 15 min. The peroxide digested material was transferred to 100 ml volumetric flask with three washings each with 5 ml double distilled water (DDW) and volume was made upto the mark.

Estimation of nitrogen

Nitrogen in plant samples was estimated by the method of Linder (1944). A 10 ml aliquot of the above digested material was taken in a 50 ml volumetric flask. To this 2 ml of 2.5 N sodium hydroxide and 1 ml of 10% sodium silicate solution were added to neutralize excess of acid and to prevent turbidity, respectively. The volume of the solution was made upto the mark with distilled water. In a 10 ml graduated test tube, a 5 ml aliquot of this solution was taken and 0.5 ml of Nessler's reagent was added. The final volume was made with distilled water. The content of the tube was allowed to stand for 5 min. for maximum colour development. The nitrogen was estimated with the help of a spectrophotometer.

Estimation of phosphorus

Total phosphorus in the material degested in sulphuric acid-peroxide was estimated by the method of Fiske and Subba Row (1925). Five ml aliquot was taken in a 10 ml graduated test tube and 1 ml of molybdic acid (2.5% ammonium molybdate in 10 N sulphuric acid) was added, followed by the addition of 0.4 ml of 1-amino 2-naphthol 4-sulphonic acid (ANSA). The colour turned blue. Volume was made upto 10 ml with distilled water. The solution was shaken for 5 min. and then transferred to colorimetric tube. The reading were taken at 620 nm on a spectrophotometer. A blank was also used simultaneously. The amount of phosphorus present in the sample was determined with the help of standard curve.

Estimation of potassium

Potassium was estimated with the help of flame photometer. After adjusting the filter for potassium in the photometer, 10 ml sulphuric acid-peroxide digested material was run. A blank was also run side by side. The amount of potassium present in sample was determined with the help of standard curve.

Analysis of micronutrients (Cu and Zn)

The analysis was done following the standard procedures (Jackson, 1973). Powdered plant material (0.5 g) was kept overnight in 5 ml of conc. HNO_3. Next day 10 ml of triacid mixture (HNO_3 : $HClO_4$: H_2SO_4, 10:4:1) was added to it for digestion. After cooling, 5 ml of 6N HCl and 50 ml distilled water was added and the contents were filtered to a 100 ml volumetric flask. This solution was directly fed to an atomic absorption spectrophotometer after caliberating the instrument with standard solutions of different dilutions (.05 ppm to 2 ppm).

Statistical analysis

The data collected were analysed statistically to determine the significance of the difference between various sample (treatment) means. All the experiments were laid out using the pattern of complete randomised block design. The data were statistically analysed by adopting appropriate mathod of 'Analysis of variance' (ANOVA) to test the null hypothesis (Ho). Statistically

significant differences between various treatment means were tested by 'least significant difference (LSD) test' at 5 and 1 per cent probability level following Dospekhov (1984).

Meteorological data

Lucknow, the capital city of the state of Uttar Pradesh where the Central Insitute of Medicinal and Aromatic Plants (CIMAP) is located has tropical monsoon type of climate. The summer season begins with April and continues till June. The onset of summer is marked by a considerable rise in temperature (Fig. 1). By the end of June, the temperature begins to fall and humidity gradually increases (Fig.2) with the arrival of humid oceanic current. The rain usually sets in by third to last week of June and continues till September (Fig.3). During the course of present experiments an unusual kind of heavy down pour in October resulted in water logging that affected the findings (details in observation and Discussion). Winter season follows the monsoon with a considerable fall of mercury and by the end of February there is a gradual increase in temperature. The figures 1-3 are based on data collected by the Meteorological Department (Govt. of India); Lucknow Headquarters.

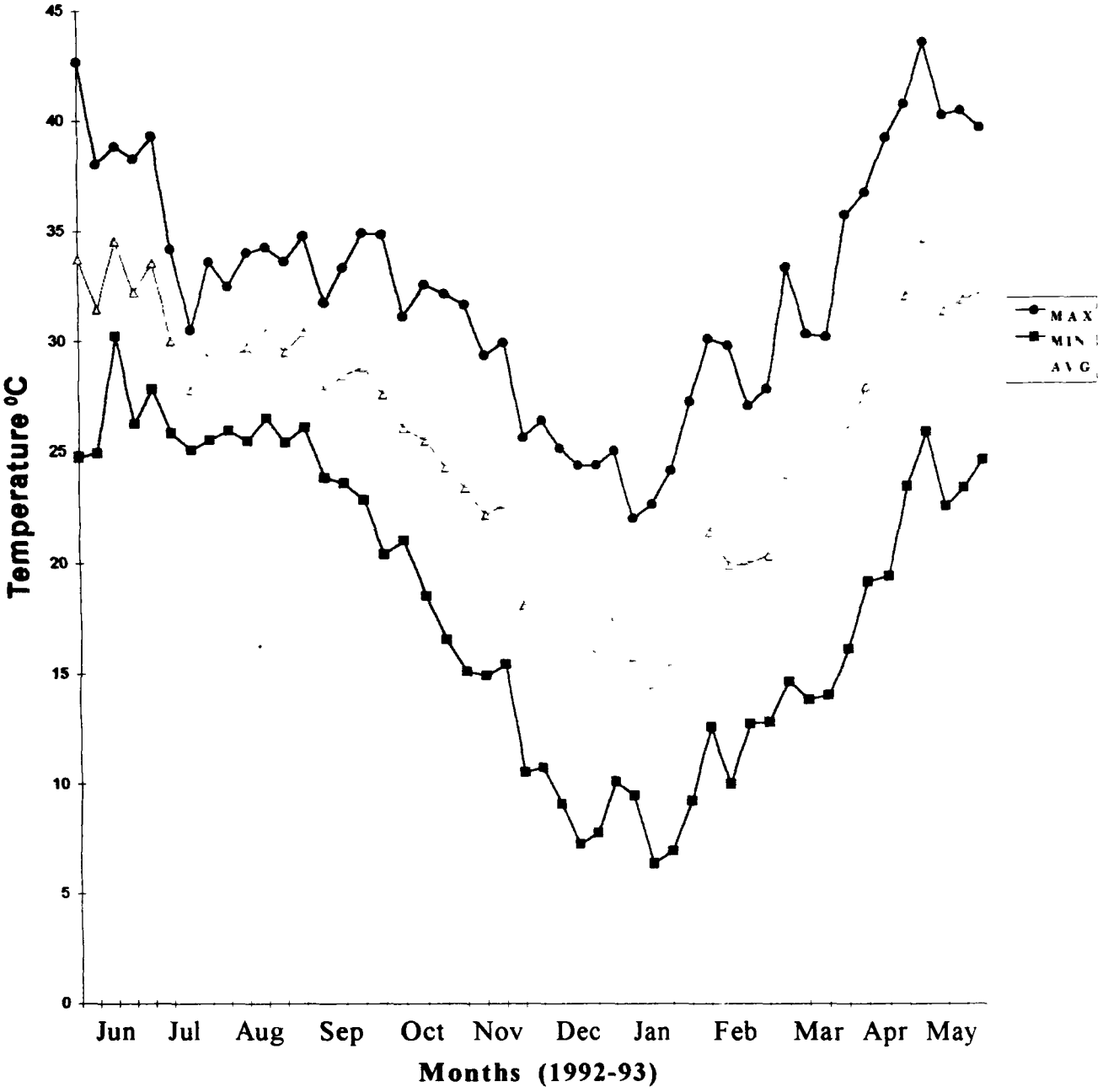


Figure 1. Maximum, minimum and average temperature during June 1992- May, 1993.

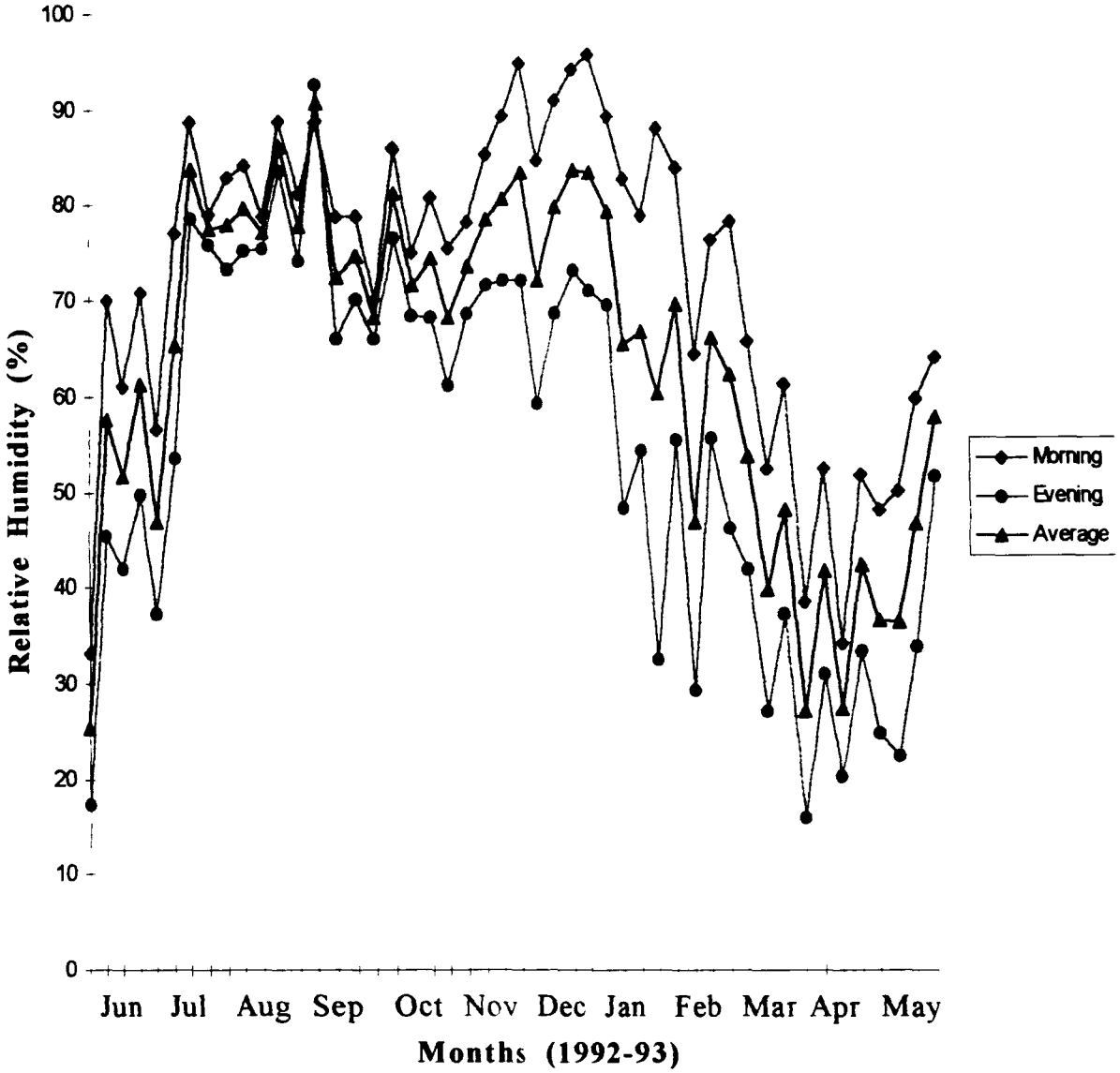


Figure 2. Morning, evening and average relative humidity during June, 1992- May, 1993.

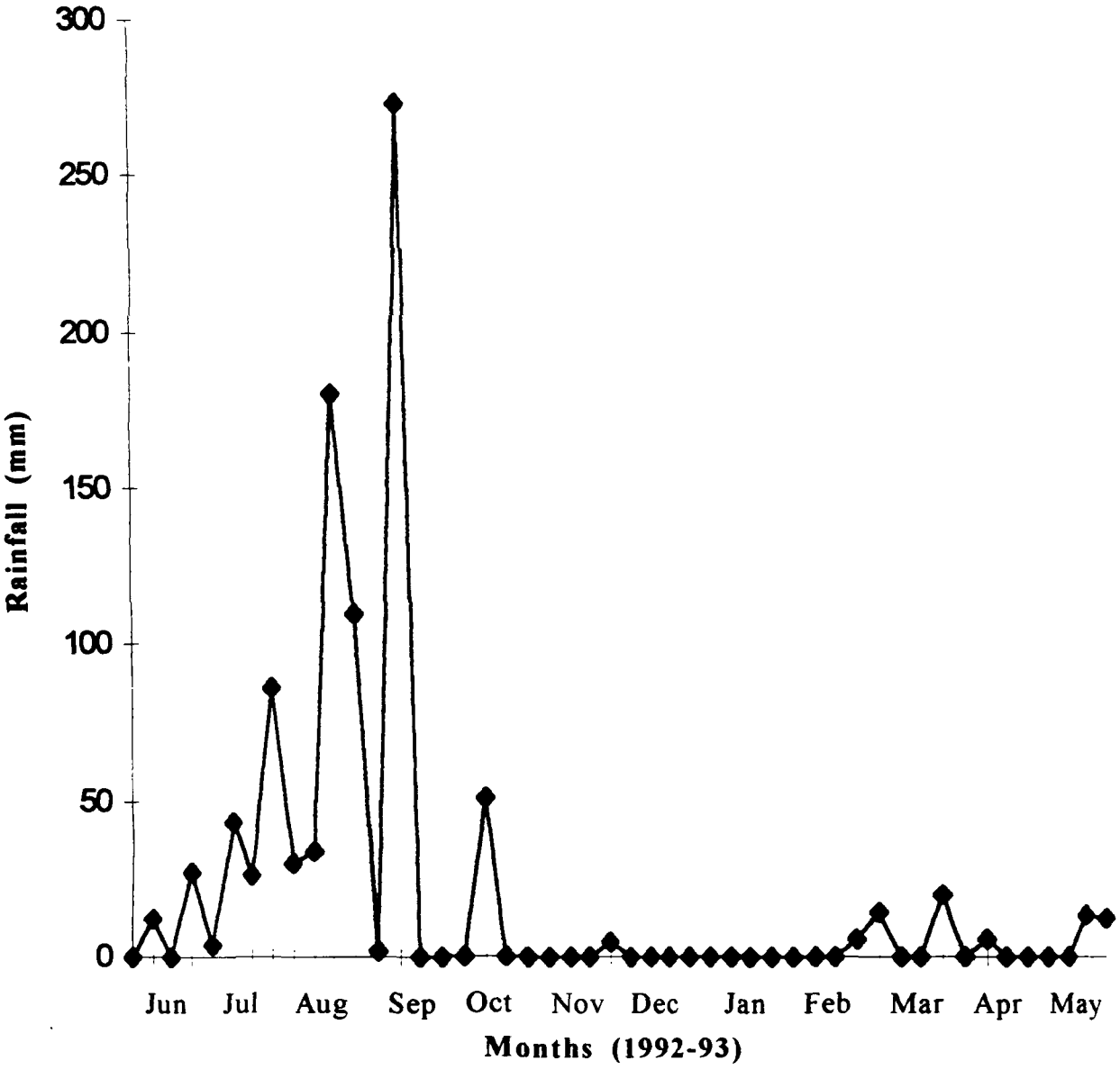


Figure 3. Rainfall during June 1992- May 1993.

OBSERVATIONS

VAM associations of *Cymbopogon* spp.

Microscopic observations of the cleared and stained root segments revealed the presence of VAM fungi in all five species of *Cymbopogon*. Roots of each species showed presence of large number of vesicles, mycelial coils and also arbuscules in their cells (Plate 1, A-D and Plate 2, A-D). The arbuscules were found to be present in lesser number as compared with the mycelial coils. The vesicles (the storage organs and distinct structure of VAM fungi) were observed to be much greater in number than arbuscules and mycelial coils.

Root colonization by VAM fungi

The roots of all selected *Cymbopogon* spp. were found to be extensively colonized by VAM fungi. The degree of VAM colonization ranged from 40 to 91.8% (Table 1). The colonization varied season to season and species to species. The *C. caesius* exhibited 54.4 to 85.2% root colonization in different seasons. The roots of *C.flexuosus* showed 52.9 to 91.84% colonization The root colonization in *C.martinii* ranged between 40 to 91.4%. However, *C.pendulus* and *C.winterianus* exhibited nearly similar ranges of root colonization i.e., 62.4 to 90.6% and 67.8 to 90.04%, respectively (Table 1).

While studying root colonization in palmarosa roots, the presence of VAM fungus (mycelium and vesicles) in the vascular cylinder was recorded

PLATE-1: Root colonization of some cultivated species of *Cymbopogon* by VAM fungi.

A&B: Root segments of *Cymbopogon caesius* showing arbuscules and mycelial coils (A) And vesicles (B).

C&D: Root segments of *Cymbopogon flexuosus* showing arbuscules and mycelial coils (C) And vesicles (D).

(Bar = 50 μm).

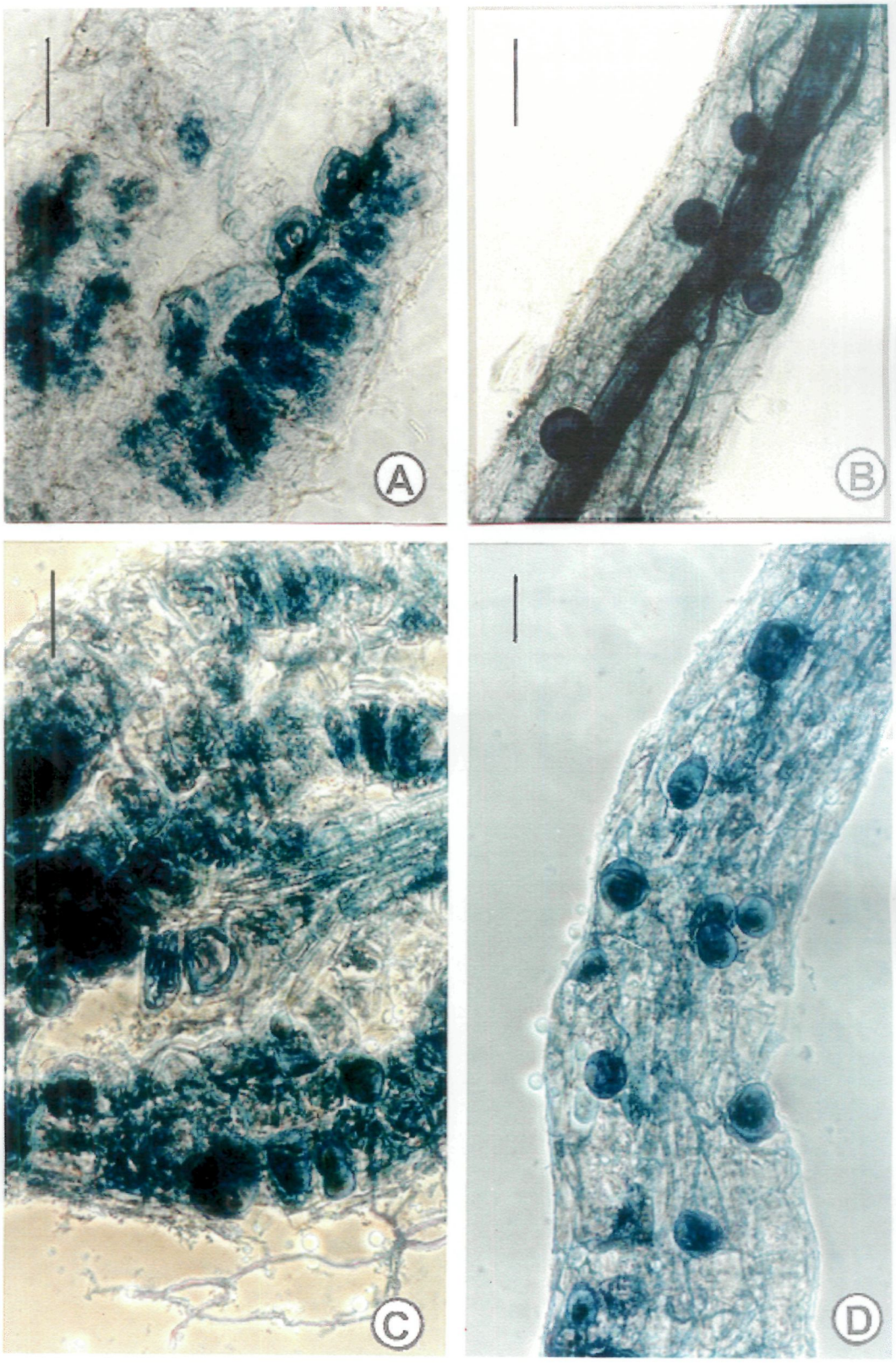


PLATE-1

PLATE-2: **Root colonization of some cultivated speices of *Cymbopogon* by VAM fungi.**

A&B: Mycelium and vesicles in the root segments of *Cymbopogon martinii* (A) and *Cymbopogon pendulus* (B).

C&D: Root segments of *Cymbopogon winterianus* showing intercalary vesicles (C) And intracalary vesicles (D).

(Bar = 50 μm).

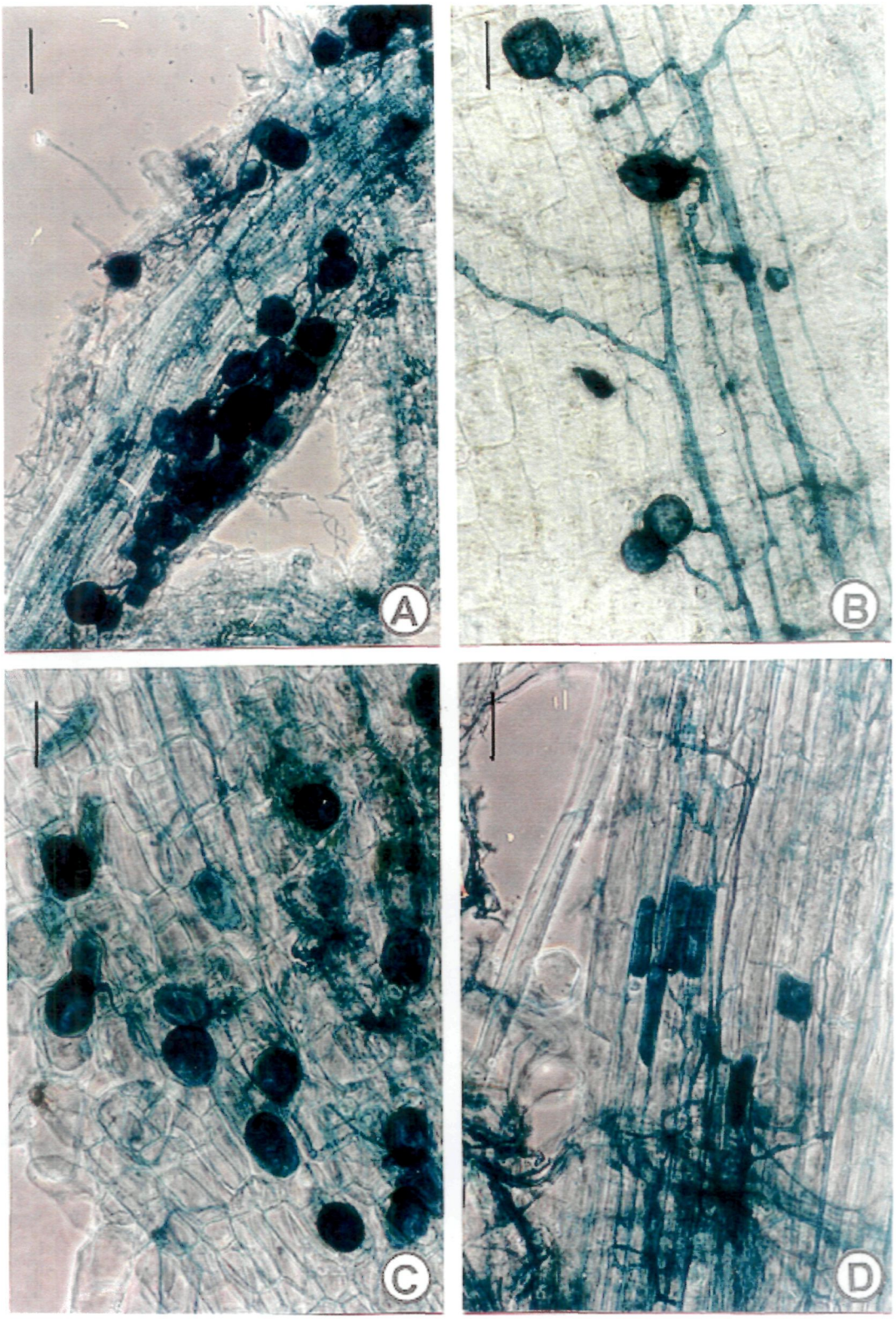


PLATE-2

Table 1. Seasonal variation in per cent root infection of *Cymbopogon* spp. by VAM fungi under field conditions

Cymbopogon spp.	Months (1992-93)												LSD	P=0.05 P=0.01
	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May		
C. caesius	85.1	77.4	83.8	88.6	70.7	70.2	65.0	58.0	54.4	57.2	65.8	78.4	8.3	
	±17.1	±8.6	±6.7	±3.1	±4.6	±4.9	±4.5	±5.7	±3.4	±4.3	±5.1	±3.6	11.1	
C. flexuosus	90.2	91.8	90.2	91.3	77.0	69.2	66.4	58.2	68.7	69.8	74.1	84.8	6.1	
	±5.7	±3.3	±5.6	±4.0	±5.6	±3.8	±5.2	±5.6	±5.2	±5.8	±4.1	±3.8	8.1	
C. martinii	91.4	80.8	75.6	79.0	76.1	81.7	77.4	68.0	63.1	74.4	40.0	65.8	7.4	
	±3.4	±6.3	±7.3	±5.6	±8.7	±4.9	±5.3	±5.8	±3.9	±4.6	±4.1	±5.4	9.8	
C. pendulus	90.6	83.0	84.7	83.2	68.4	70.7	68.2	78.2	78.6	64.8	62.4	82.6	5.0	
	±4.9	±3.5	±3.4	±5.8	±5.1	±4.7	±4.7	±4.8	±6.9	±5.6	±5.2	±4.0	6.7	
C. winterianus	92.5	84.8	75.2	89.6	87.4	72.4	74.2	85.4	82.2	67.8	80.8	83.2	10.6	
	±5.5	±7.2	±4.8	±7.7	±3.4	±4.9	±4.3	±5.4	±6.7	±6.0	±5.5	±4.92	14.2	

Mean ± standard deviation

(Plate 3 A-D). The infection was noticed in and around the pits of vascular tissues (Plate 3A, B). The mycelia were found to occur along the lateral walls of the axially arranged vascular cells as well as on the end walls (Plate 3A, B). In transverse section of the root, infections were noticed in some thin-walled endodermal cells (Plate 4 B-1), pericycle (Plate 4 A-2), axial parenchyma, protoxylem and large metaxylem (Plate 4B-2, C-1,D-1), including the pith cells (Plate 4A-1). It was also found to be present at plasmodesmatal sites between the two parenchyma as well as between parenchyma and other adjacent vascular tissues (Plate 4B-3).

VAM fungi in the rhizosphere

VAM spore count in the rhizosphere of *Cymbopogon* spp. ranged from 12 to 47 spores per gram soil . The rhizosphere of *C.martinii* had minimum spore count (12 to 36 spores per gram soil). The spore counts, however, varied from season to season and with the species of *Cymbopogon.* Largest VAM spore count (14 to 47 per gram soil) was found in the rhizosphere of *C. winterianus*. The rhizosphere soil of *C.caesius* was observed to have 13 to 38 spores per gram of soil, *C.flexuosus* 14 to 43 spores per gram soil, and *C. pendulus* 14 to 41 spores per gram soil (Tabel 2).

Identification of VAM fungi

Microscopic examination revealed that various kinds of spores and sporocarps were present in the rhizosphere soil of *Cymbopogon* plants. The spores were found to be of different genus and species of VAM fungi. The soil

PLATE-3: Colonization of the vascular tissues of *Cymbopogon martinii* roots.

A: Mycelium and intracalary vesicles.

B: Mycelium and intracalary vesicles along the root axis.

C: Intercalary and intracalary vesicles

D: Macerated vascular elements showing intracalary vesicles.

(Bar = 20 μm).

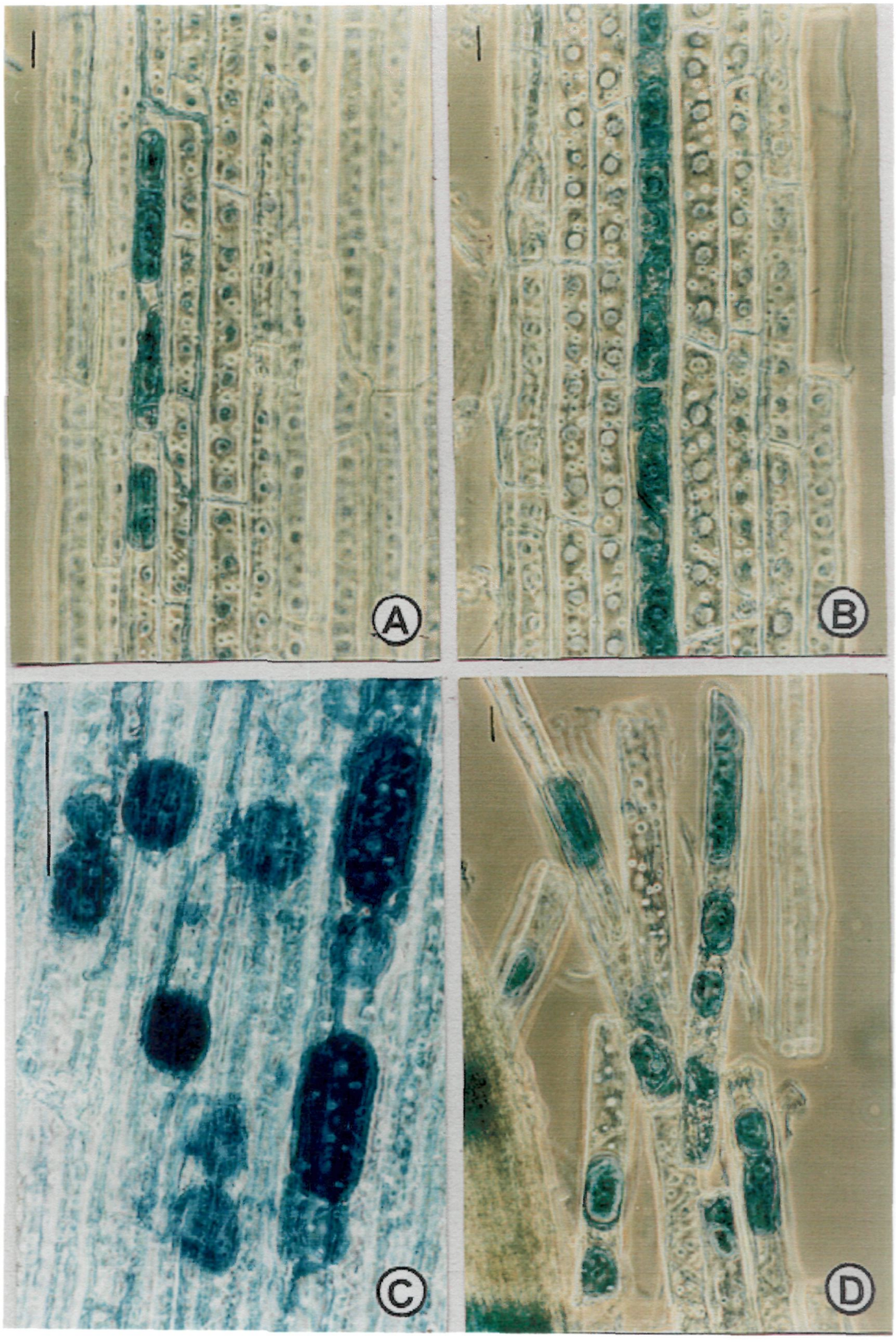


PLATE-3

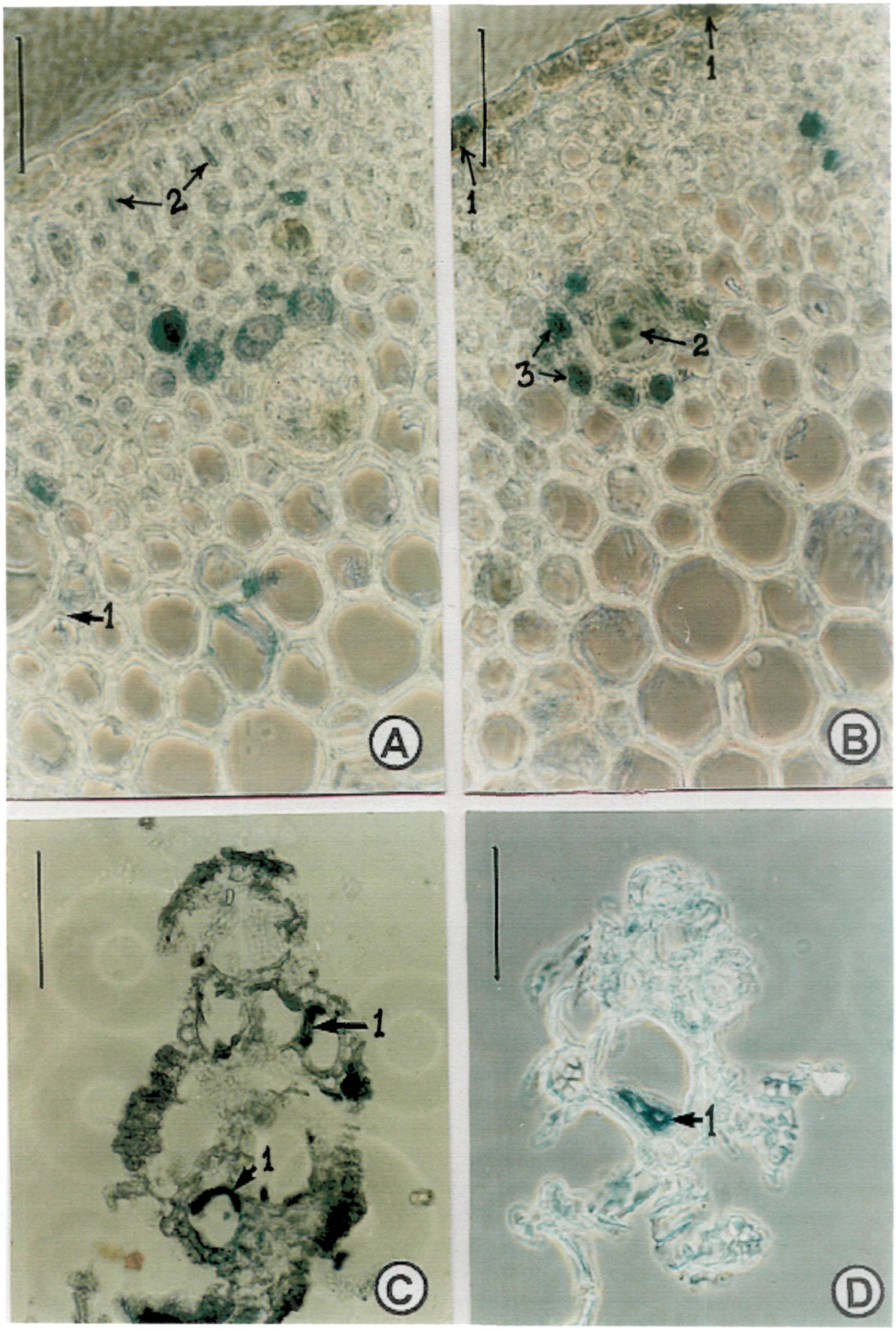


PLATE-4

Table 2. Seasonal variation in spore count of VAM fungi (g^{-1} soil) in the rhizosphere of *Cymbopogon* Spp. under field conditions.

	Months (1992-93)													
Cymbopogon Spp.	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	LSD	P=0.05 P=0.01
C. caesius	15±.4	14±.4	15±.4	17±2	13±.2	23±.4	23±.5	23±.3	38±.7	27±.5	23±.4	20±.4	0.56 0.75	
C. flexuosus	17±.3	14±.4	14±.3	22±.4	14±.3	32±.3	32±.5	32±.4	43±.3	36±.4	27±.3	20±.3	0.48 0.64	
C. martinii	14±.2	14±.3	14±.2	19±.2	12±.2	20±.4	25±.5	30±.2	35±.4	34±.7	24±.4	21±.4	0.48 0.64	
C. pendulus	18±.3	14±.3	15±.3	23±.3	14±.4	31±.5	31±.5	27±.5	41±.5	31±.7	25±.6	25±.4	0.57 0.76	
C. winterianus	17±.5	20±.4	15±.3	19±.4	14±.4	28±.5	29±.5	29±.4	40±.2	47±.5	33±.3	21±.4	0.51 0.69	

Mean ± standard deviation

predominantly had spores belonging to several species of *Glomus*. The mycorrhizal species were identified on the basis of morphological characters of spores. The spores and sporocarps of *Glomus aggregatum, G. fasciculatum, G. mosseae* and spores without sporocarps of *G. geosporum*, and *G. reticulatum* were relatively in high numbers. Spores of *G. dimorphicum, G. macrocarpum, G. multicaule, G. occultum* and a *Gigaspora* sp. were very few in number. Morphological characters of these species are as follows:

Glomus aggregatum Schenck and Smith emend-Koske

Sporocarpic species, spores loosely aggregated in sporocarp without peridium, spores globose to subglobose, 55-160x60-160 µm, very pale yellow to yellow brown, spore wall double layered, walls separable, wall thickening 2-4 µm, subtending hypha generally straight and pores open (Plate 5 B,C).

Glomus dimorphicum Boyetchko and Tewari

Sporocarp not seen, spores found singly in soil, globose to subglobose, 95-270 µm, yellow to reddish brown, spore wall three layered, organised in two wall groups. Hyaline, laminated first wall, 2-8 µm thick, sloughed off with age. Light yellow second wall became reddish brown at maturity, laminated, 2-8 µm. Membranous third wall, light yellow to reddish brown, wrinkled in certain places, 1 µm thick. Straight or slightly curved subtending hypha, light yellow to light brown, attached occasionally with a septum, often occluded, another septum often present at a distance from the point of attachment (Plate 5 D).

PLATE 5: Spores and sporocarps of VAM fungi (A-G) isolated from rhizosphere soil of cultivated *Cymbopogon* spp.

A : *Gigaspora* sp.

B : Sporocarp of *Glomus aggregatum*

C : Single spore of *G. aggregatum*

D :*G. dimorphicum*

E : A portion of sporocarp of *G. fasciculatum*

F : *G. fasciculatum* spores

G : A spore of *G. fasciculatum* showing ingrowths of wall.

(Bar = 50 μm).

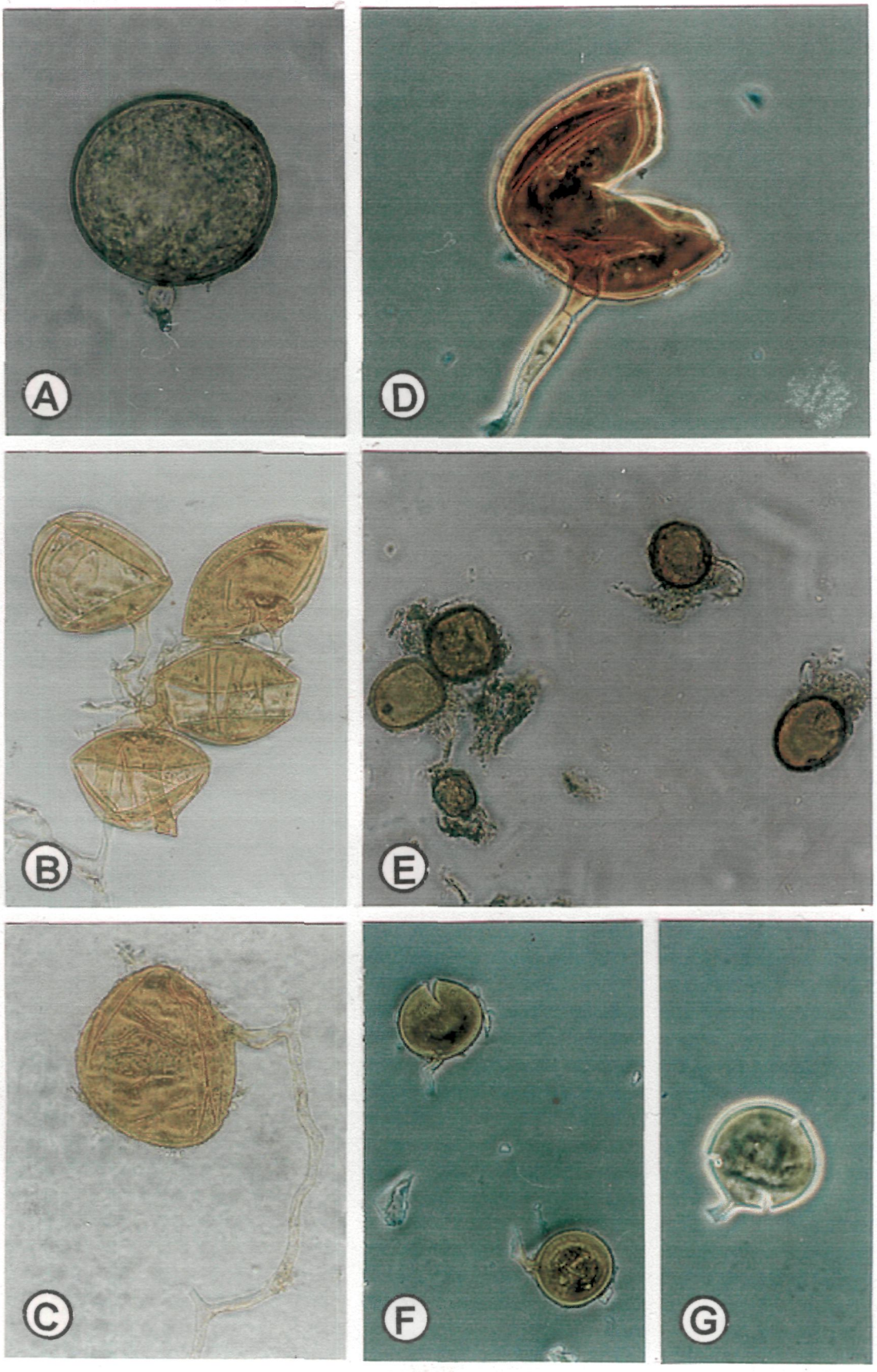


PLATE - 5

Glomus fasciculatum (Thaxter) Gerdemann and Trappe emend. Walker and Koske.

Sporocarpic species but intact sporocarp could not be recovered. Broken sporocarp had no sign of peridium. Chlamydospores were also found free in soil, in loose aggregations and in clusters as well. Spores globose to subglobose, sometimes irregular, 36-70 x 40-90 μm, light yellow to yellow brown in colour; spore wall three layered with a total thickness 4 μm, outer and inner wall thin and hyaline. Middle thicker wall pale yellow to yellowish brown in colour and relatively thicker at the junction of subtending hyphae with a gradual decrease in thickness in subtending hyphae (Plate 5 F,G). Pores usually open.

Glomus geosporum (Nicolson and Gerdemann) Walker

Sporocarp not seen, chlamydospores found singly in soil. Shape globose to subglobose, size varied between 100-160 x 105-184 μm, light yellow brown to dark yellow brown in colour. spore wall three layered with a total thickness of 6-10 μm. Outer and inner layers thin and hyaline and middle layer thicker and yellow brown to red brown. Generally straight and slightly funnel shaped subtending hyphae, the wall thickness extending upto 80 μm along the hyphal wall from the spore base (Plate 6 A).

Glomus macrocarpum Tul. and Tul.

Sporocarp not seen, chlamydospores found singly in soil, golden

PLATE 6 : **Spores of VAM fungi isolated from rhizosphere soil of cultivated** *Cymbopogon* **spp.**

A : *Glomus geosporum*

B : *G.macrocarpum*

C : *G.mosseae*

D : *G.multicaule*

E : *G.occultum*

F : *G.reticulatum*

(Bar = 50 μm).

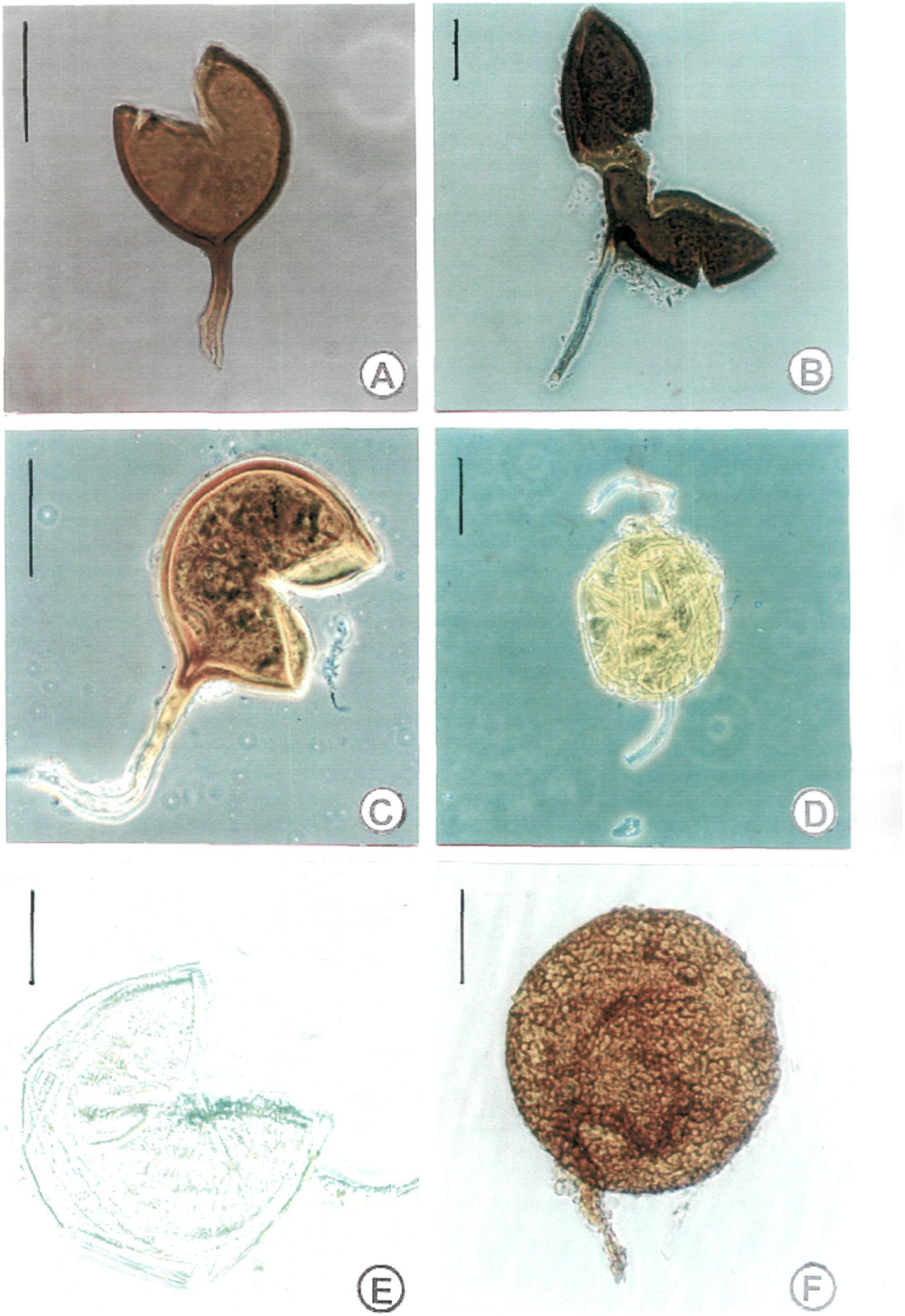


PLATE-6

brown in colour, globose to subglobose in shape and, 100-125 x 90-110 µm in size. Spore wall two layered. Outer layer thin and hyaline, inner layer thick and yellow coloured. The wall thickening continued into the subtending hypha upto 50µm. Spores with hyaline and straight subtending hyphae (Plate 6 B).

Glomus mosseae (Nicolson and Gerdemann) Gerdemann and Trappe

Sporocarpic species, spores yellow to yellow brown in colour, globose to ovoid in appearance and 110-300 x 120-330 µm in size. Funnel shaped base, divided from subtending hyphae by curved septum . Spores wall two layered, 4 µm thick, outer layer thin and hyaline and inner layer thick and brownish yellow. (Plate 6-C).

Glomus multicaulis Gerdemann and Bakshi

Chlamydospores were found singly in soil. Spores golden brown to dark brown in colour, 150-230 x 130-155 µm in size and, ellipsoidal or subglobose in shape, with 1-2 hyphal attachments. Spores with two hyphal attachments, attached on opposite ends (Plate 6 D). Spore wall 10-25 µm thcik with rounded projections regularly distributed all over the inner surface.

Glomus occultum Walker

Clourless, hyaline chlamydopores found singly in soil. Spores subangular in outline, 25-90 x 30-115 µm in size having hyaline and oily content. Two layered spore wall with an additional deposition of granular material on outer surface. Outer layer thin and inner layer of spore wall bilaminate. Simple subtending hypha, generally recurved and attached

accentrically (Plate 6 E).

Glomus reticulatum Bhattacharjee and Mukerji

Chlamydospores found freely and singly in the soil. Spores golden brown to dark brown in colour, globose in shape and 140-170 μm in diameter with regular reticulate markings on walls. Spores wall 12-15 μm thick, differentiated into two layers, outer layer fissured and inner layer with reticulate markings. Subtending hypha funnel shaped, 9-10 μm wide at attachment point with open pore(Plate 6 F).

Gigaspora Gerdemann and Trappe

Chlamydospores found singly in the soil. Spores globose to subglobose in shape, light yellow in colour and 215-300 μm in diameter. Spores borne on a bulbous suspensor like cell, with a narrow hyphae extending from a peg like projection towards the spore. Thin spore walls, two in number, μm thick(Plate 5 A).

Seasonal variarion of VAM fungi

The trend in VAM spore counts in the rhizosphere soils of all the five *Cymbopogon* spp. during year 1992-93 was similar. In June 1992, spore counts in the rhizosphere soils of the different *Cymbopogon* spp. varied from 15-18. The spore count declined till August in all species except in *C. winterianus*. In this species, the spore count increased in July and then declined in August. The VAM spore number in the rhizosphere soil of all

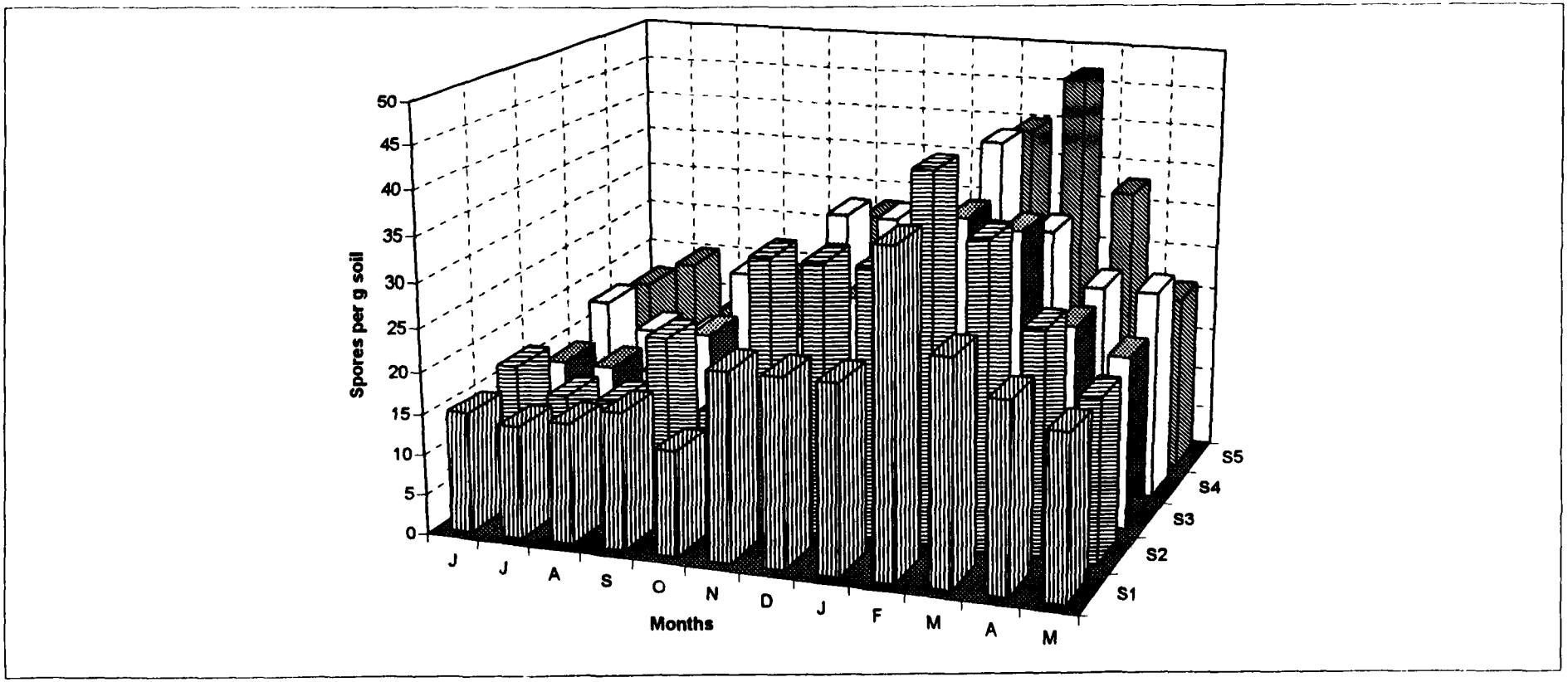


Figure 4. VAM spore count in the rhizosphere of five *Cymbopogon* spp. in 12 calendar months.
S1=*Cymbopogon caesius*, S2=*C. flexuosus*, S3= *C.martinii*, S4=*C.pendulus*, S5= *C.winterianus.*

Cymbopogon spp. showed an upward trend from the month of September onwards (Fig. 4) with decrease in average temperature (Fig.1). During November, December, 1992 and January, 1993 the spore count remained almost same in rhizosphere of all the five *Cymbopogon* spp. (Fig. 4) except in *C. martinii* where the spore count showed an upward trend. The maximum count was observed in the month of February (Fig. 4) with average temperature 19.8°C (Fig.1) in all the *Cymbopogon* spp. (except *C. winterianus*). In *C. winterianus* maximum spore count was obverved in March. From March (in *C. winterianus* from April) spore count again declined with increase in average temperature. Minimum spore count was recorded in October (Fig. 4) when water logging condition was also observed.

The VAM fungus did not show any marked trend in infecting *Cymbopogon* roots and also they did not behave similarly in all *Cymbopogon* spp. under study (Fig. 5). However,a heavy infection was recorded in the month of June in all selected *Cymbopogon* spp. except in *C. flexuosus* In the latter species, infectivity got strength only in July. Minimum root infection was found in the months February (*C. caesius* and *C. flexuosus*), March (*C. winterianus*) and April (*C. pendulus and C. martinii*).

The root colonization did not show any correlation with spore count in the rhizophere. The maximum spore count was recorded in the month of February in all *Cymbopogon* spp. except *C. winterianus* (in March) where as maximum root infection was found in June (Fig. 5). Low spore sount was found in July, August and October where as root infection was low in months

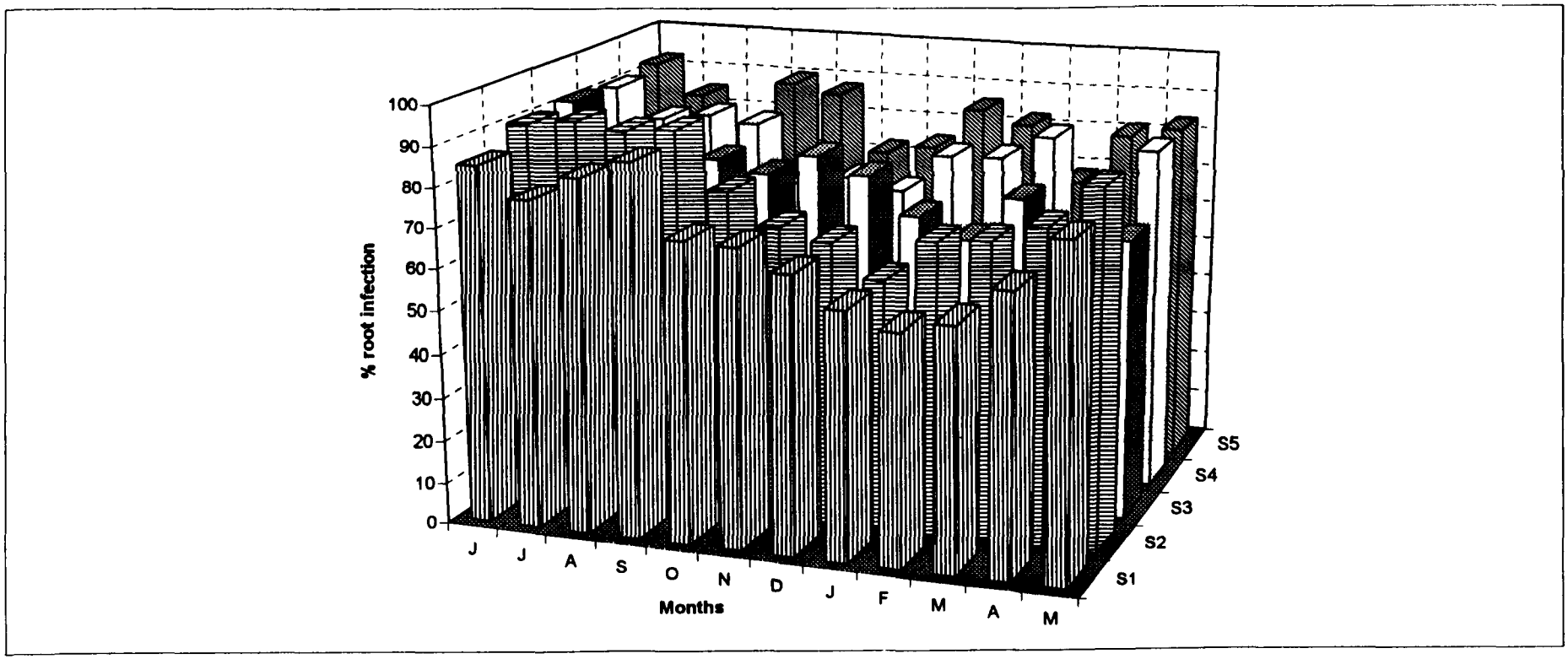


Figure 5. VAM colonization of roots of five *Cymbopogon* spp. in 12 calendar months. S1=*Cymbopogon caesius*, S2=*C. flexuosus*, S3= *C.martinii*, S4=*C.pendulus*, S5= *C.winterianus*.

of February, March and April.

Impact of VAM fungi on growth and productivity of *Cymbopogon* spp.

Inoculation of all the three *Glomus* spp. (*G.aggregatum*, G. *fasciculatum*, G.*mosseae*) either singly or in combination imparted favourable effect on plant growth of all the three *Cymbopogon* spp. (*C. flexuosus*, *C. martinii*, C. *winterianus*). Marked increase was observed in all the parameters of the inoculated plants (Tables 3,4,5; Fig. 6).

Effect of VAM inoculation on *Cymbopogon flexuosus*

Cymbopogon flexuosus plants inoculated with either of the three VAM fungi or their combinations showed marked increase in plant height, tillering and shoot fresh weight as compared to non-mycorrhizal (control) plants (Table3, Plate 7). Maximum increase (26%) in plant height was obtained by the inoculation with *Glomus aggregatum* and minimum with *G.mosseae* (6%). Treatment with *G. fasciculatum* and mixture of three species caused 18% and 10% increase, respectively. Plant tillering was also increased significantly due to the VAM treatements. The maximum tillering was brought about by the inoculation of *G. fasciculatum* (Fig. 6). The impact of other three treatment did not differ significantly although the increases were significantly greater than control plants. The shoot fresh weight of incoculated plants also increased and ranged between 175.8 to 242 g compared to 162 g control plants. Maximum increase in shoot fresh weight (49%) was obtained by the

Table 3. Performance of *Cymbopogon flexuosus* (lemon-grass) treated with three different species of *Glomus*.

Treatments	Growth parameters			VAM associations	
	Plant height (cm)	Tillers (number)	Shoot fresh wt. (g)	Root colonization (%)	VAM spore count (spores g^{-1}soil)
Control	138.5 ± 2.9	13 ± 1.3	162.0 ± 4.7	–	–
Glomus aggregatum	174 ± 3.8 (26)*	17 ± 1.72 (31)	42.0 ± 5.1 (49)	88.6 ± 2.1	22 ± 0.5
Glomus fasciculatum	163.2 ± 3.0 (18)	19 ± 0.9 (46)	222.4 ± 4.6 (37)	90.0 ± 1.9	12 ± 0
Glomus mosseae	146.4 ± 2.5 (6)	16 ± 0.9 (23)	175.8 ± 2.5 (9)	82.0 ± 6.0	17 ± 0.5
Mixture of above three spp.of *Glomus*	152.2 ± 4.7 (10)	15 ± 1.0 (15)	198.0 ± 8.5 (22)	87.0 ± 4.6	20 ± 0.5
LSD P=0.05	2.12	1.5	1.23	4.52	0.44
P=0.01	2.92	2.0	9.96	6.34	0.61

Mean ± standard deviation; * values within parentheses represent per cent increase over control.

PLATE 7 : Plant growth of *Cymbopogon flexuosus* inoculated with different species of VAM fungi ; i = inoculated, c= control.

A : *Glomus aggregatum*

B : *G. fasciculatum*

C : *G. mosseae*

D : Mixture of all three spp. (A+B+C).

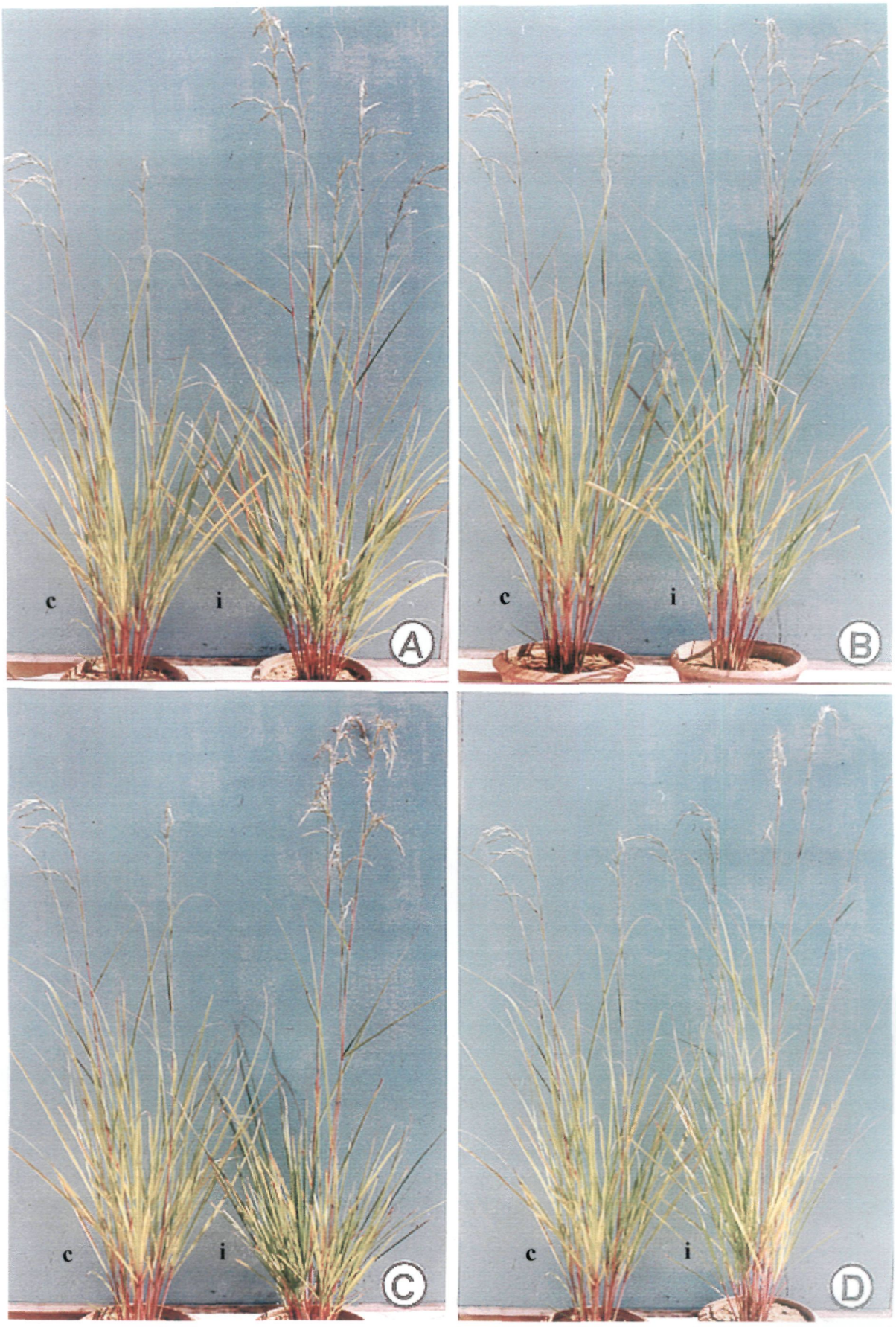


PLATE NO. 7

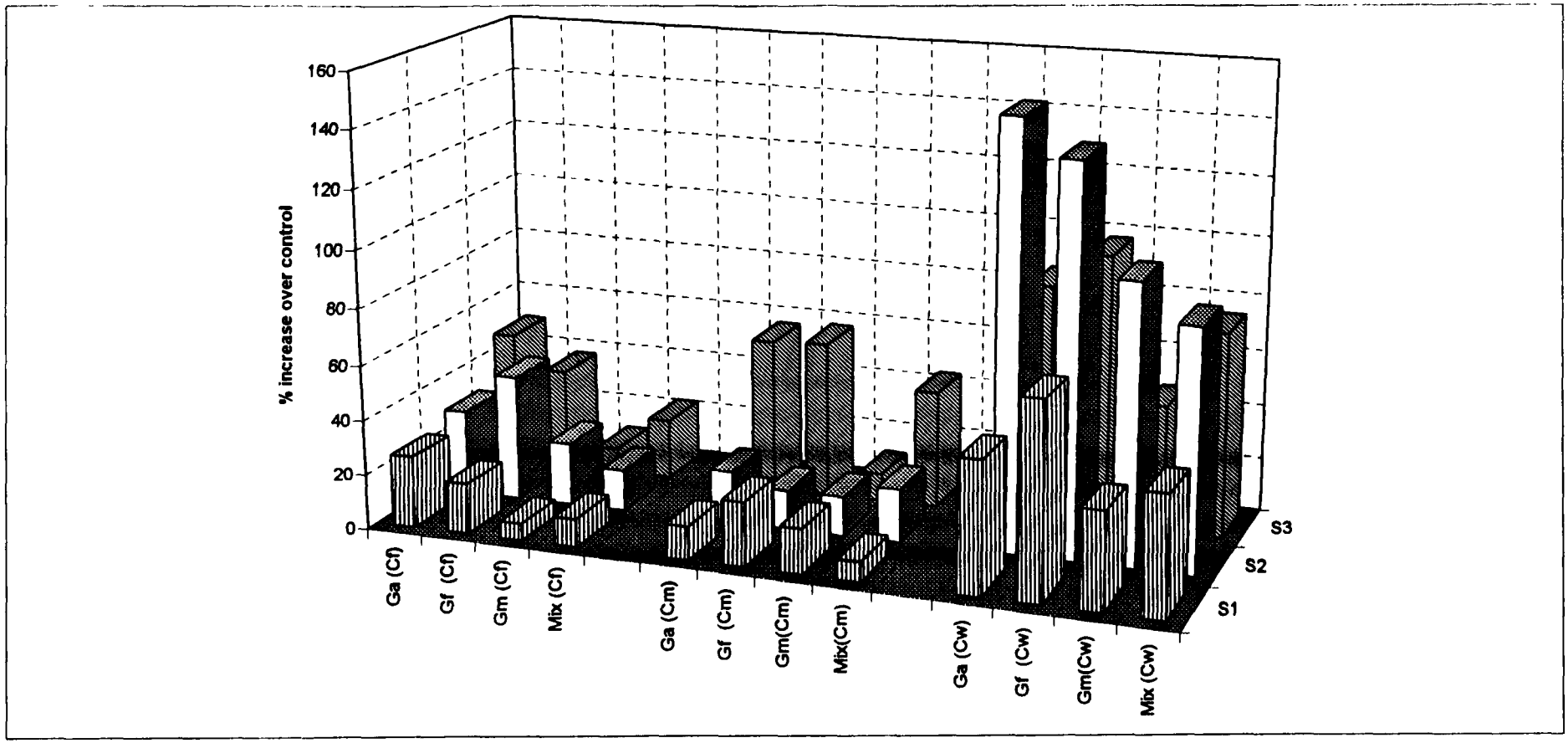


Figure 6. Effect of VAM inoculation on growth performance of three selected *Cymbopogon* spp.
S1 = Plant height, S2 = Number of tillers, S3 = Shoot fresh weight (% increase)
Cf = *Cymbopogon flexuosus* , Cm = *C.martinii*, Cw=*C.winterianus*, Ga=*Glomus aggregatum*, Gf=*G.fasciulatum*, Gm=*G.mosseae*, Mix=mixture of three *Glomus* spp.

treatment with *G. aggregatum*.. Inoculation of *G. fasciculatum* and mixture of the three species brought about 37% and 22% increase respectively. *G. mosseae* was least effective in increasing the fresh weight (Fig. 6).

Chlamydospore count in the soil did not show any correlation with the root colonization. Root colonization by the VAM fungi was fairly high. Root colonization in plants inoculated with *G. fasciculatum* was 90% and with *G. mosseae* 82%. Spore count was highest in plants treated with *G. aggregatum* and lowest with *G. fasciculatum* (Table 3). Spore count ranged between 12 to 22 spores g^{-1} soil.

Effect of VAM inoculation on *Cymbopogon martinii*

VAM inoculated *C. martinii* plants gave better response than inoculated *C. flexuosus* plants. A significant increase in plant height occurred in all the inoculated plants as compared to uninoculated control plants. *G. fasciculatum*, was most effective where an increase of 23% over control occurred. With mixture of the VAM species, increase in plant height was only 7%. The plant height of inoculated plants ranged between 176.8 cm to 203.4 cm compared 165.8 cm of control plants. VAM inoculation also caused significant increase in plant tillering. The impact caused by different VAM fungi on plant tillering, however, did not differ significantly. Shoot biomass also significantly increased. The increase ranged from 10 and 57% over their respective controls. *G. aggregatum* and *G. fasciculatum* were most effective as they caused 56 and 57% increase, respectively. The effect of *G. mosseae* was lowest as only 10% was observed (Table 4, Plate 8).

Table 4. Performance of *Cymbopogon martinii* (palmarosa) treated with three different species of *Glomus* .

Treatements	Growth Parameters			VAM associations	
	Plant hight (cm)	Tillers (numbers)	Shoot fresh wt (g)	Root colonization (%)	VAM spore count (spores g^{-1}soil)
Control	165.8 ± 2.9	20 ± 1.6	162.6 ± 4.28	–	–
Glomus aggregatum	185.8 ± 3.0 (12)*	26 ± 3.3 (19)	254.4 ± 3.97 (56)	83.0 ± 3.5	22 ± 0
Glomus fasciculatum	203.4 ± 7.6 (23)	24 ± 2.6 (14)	255. 6 ± 3.21 (57)	90.0 ± 5.8	13 ± 0.5
Glomus mosseae	192.0 ± 3.4 (16)	24 ± 2.6 (14)	179.6 ± 5.94 (10)	78.4 ± 3.8	21 ± 0
Mixture of above three spp. of *Glomus*	176.8 ± 21.3 (7)	26 ± 2.5 (19)	232.8 ± 3.56 (43)	88.8 ± 1.6	26 ± 0
LSD P=0.05	13.07	3.6	5.58	2.76	0.38
P=0.01	18.0	4.9	7.69	3.88	0.53

Mean ± standard deviation; * values within parentheses represent per cent increase over control.

PLATE 8 : Plant growth of *Cymbopogon martinii* inoculated with different species of VAM fungi ; i = inoculated, c= control.

A : *Glomus aggregatum*

B : *G. mosseae*

C : *G. fasciculatum*

D : Mixture of all three spp. (A+B+C).

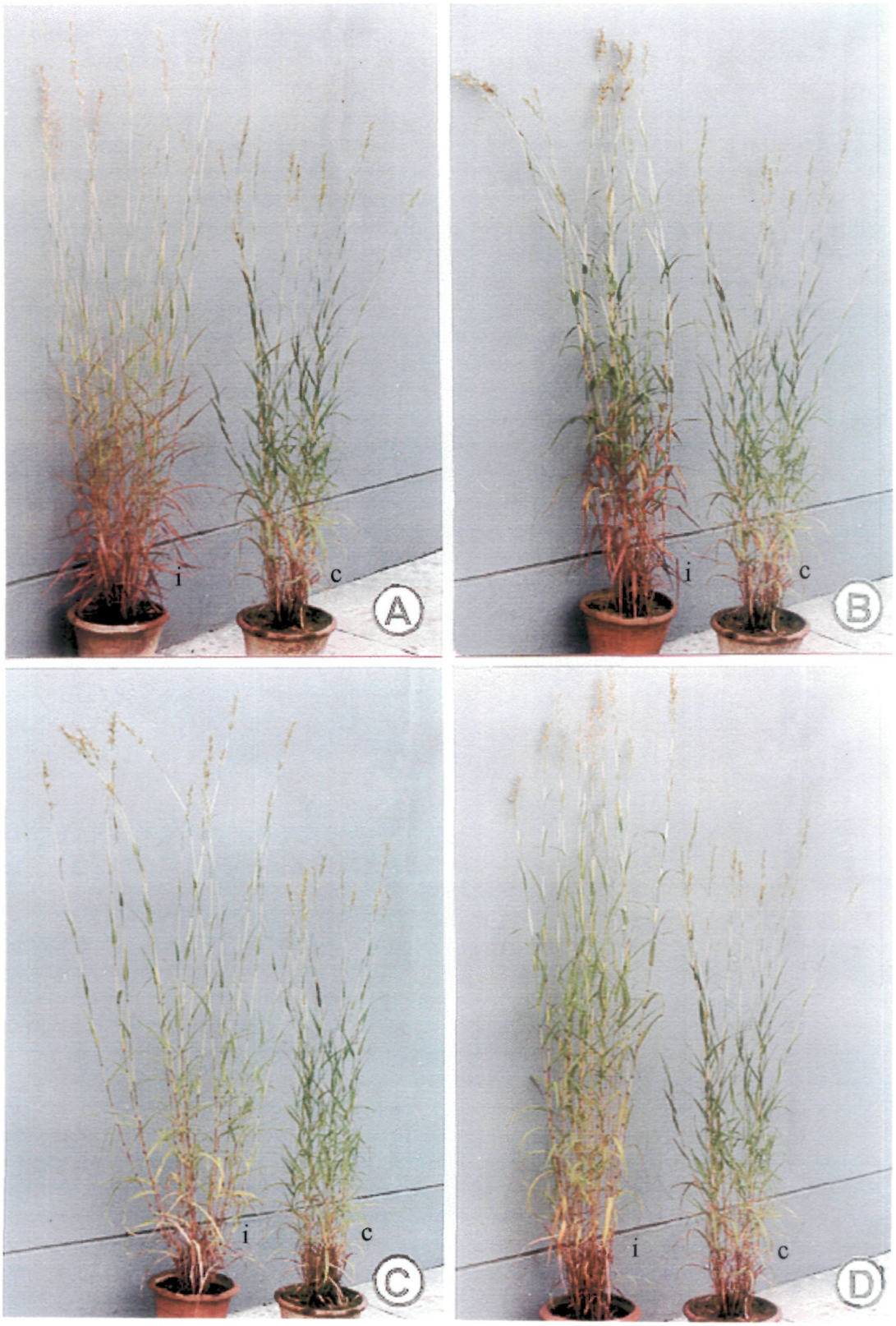


PLATE-8

Root colonization of *C. martinii* by the VAM fungi ranged between 78.0% and 90% being considerably high by *G. fasciculatum* (90%) and relatively low by *G. mosseae* (78 %). The root colonization was directly correlated with the increase is shoot biomasss as plants inoculated with *G. fasciculatum* showed highest increase in shoot biomass and root colonization and the lowest increase in shoot biomass and root colonization occurred with *G. mosseae*. Root colonization and spore count in the rhizospere did not show any correlation. The spore number (26 spore g^{-1} soil) was highest in the rhizosphere of plants inoculated with mixture of the species and minimum (13 spores g^{-1} soil) with *G. fasciculatum* (Table 4).

Effect of VAM inoculation on *Cymbopogon winterianus*

Plants of *C. winterianus* treated with different VAM spp. responded more favourably than other two *Cymbopogon* spp. The plant height of all the inoculated plants significantly increased (34 to 70%) compared to control plants. VAM inoculation also significantly increased tillering (87-150%). The *G. aggregatum* and *G. fasciculatum* were equally effective. Their impact on tillering was better than *G. mosseae* and the mixture of the VAM species. Shoot biomass production was also enhanced (56-97%). *C. winterianus* with *G. fasiculatum* caused highest enhancement. *G. mosseae* was least effective. *G. fasciculatum* increased shoot fresh weight by 97% and plant height by 70% which was highest among the VAM species. *G. mosseae* caused only 34% increase in plant height and 56% in shoot fresh weight. Impacts of *G. aggregatum* and mixture of VAM species were similar (Table 5, Plate 9).

Table 5. Performance of *Cymbopogon winterianus* (citronella) treated with three different species of *Glomus*

Treatements	Growth Paramenters			VAM associations	
	Plant height (cm)	Tillers (number)	Shoot fresh wt (g)	Root colonization (%)	VAM spore count (spores g^{-1}soil)
Control	81 ± 4.9	8 ± 1.1	86 ± 1.58	–	–
Glomus aggregatum	119 ± 3.7 (47)*	20 ± 1.8 (150)	159.0± 2.24 (85)	93.2 ± 0.8	21 ± 0.5
Glomus fasciculatum	137.6 ± 3.6 (70)	19 ± 1.5 (137)	169.4 ± 4.36 (97)	88.8 ± 3.9	7 ± 0.0
Glomus mosseae	108.8 ± 4.5 (34)	16 ± 1.2 (100)	134.0 ± 3.39 (56)	87.6 ± 3.2	19 ± 0.5
Mixture of above three spp. of *Glomus*	115.4 ± 3.8 (42)	15 ± 1.0 (87)	149.4 ± 4.36 (74)	91.0 ± 4.0	20 ± 0.0
LSD P=0.05	6.12	1.6	4.77	4.16	0.52
P=0.01	8.41	2.2	6.57	6.47	0.73

Mean ± standard deviation; * values within parentheses represent per cent increase over control.

PLATE 9 : Plant growth of *Cymbopogon winterianus* inoculated with different species of VAM fungi ; i = inoculated, c= control.

A : *Glomus aggregatum*

B : *G. fasciculatum*

C : *G. mosseae*

D : Mixture of all three spp. (A+B+C).

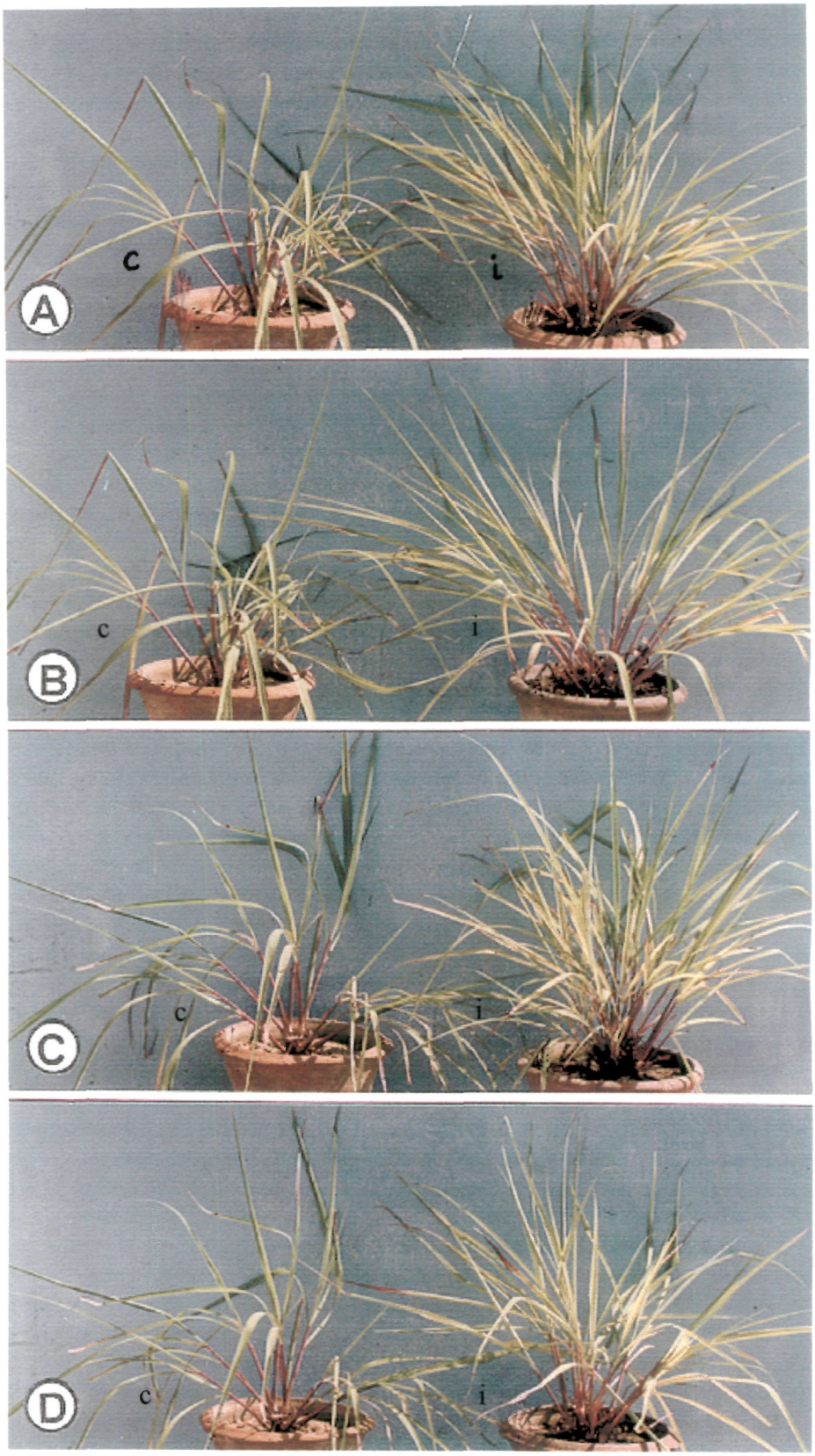


PLATE -9

Roots of the inoculated plants of *C. winterianus* showed intensive colonization by the VAM fungi in all the treatments. Spore count for *G. aggregatum* was highest (21 spores g^{-1} soil) and for *G. fasciculatum* (7 spores g^{-1} soil) lowest. Root colonization and spore counts did not show any correlation with the impacts of VAM fungi on growth performance of plants (Table 5).

Effect of VAM fungi on quality and quantity of essential oils

All the three species of *Cymbopogon* viz. *C. flexuosus* (lemon-grass), *C. martinii* (palmarosa) and *C. winterianus* (citronella) showed an increase in their essential oil contents when inoculated with the VAM fungi, compared to uninoculated plants (Table 6, Fig. 7).

Cymbopogon flexuosus (lemon-grass)

Cymbopogon flexuosus showed an increase in essential oil content due to mycorrhization. The increases were caused by either of the VAM fungus however, not significant statistically. Among VAM fungi tried, *G. fasciculatum* caused highest increase (17%) in oil content . The mixture of the VAM fungi also failed to increase oil content in lemon-grass (Fig. 7). The citral content of lemon-grass oil ranged between 84.7 to 87.2 % as compared to 83.7 % in control (Table 6).

Cymbopogon martinii (palmarosa)

The VAM inoculated plants of palmarosa showed a significant increase in their essential oil content. The increase ranged between 31-69 %. The

Table 6. Effect of mycorrhization on quantity and quality of essential oils of selected *Cymbopogon* spp.

Treatments	*C. flexuosus* (Lemon-grass)		*C. martini* (palmarosa)		*C. winterianus* (citronella)	
	Oil Content	Citral*	Oil Content	Geraniol*	Oil Content	Citronellal*
Control	0.54 ± 0.05	83.71 ± 0.21	0.64 ± 0.1	80.50 ± 1.18	1.4 ± 0.14	26.73 ± 0.38
Glomus aggregatum	0.62 ± 0.07 (15)*	84.67 ± 0.4 (1)	1.08 ± 0.3 (69)	83.57 ± 0.99 (3)	2.17 ± 0.07 (55)	28.24 ± 0.39 (6)
Glomus fasciculatum	0.63 ± 0.06 (17)	86.47 ± 0.93 (3)	0.84 ± 0.6 (31)	81.47 ± 1.86 (1)	2.04 ± 0.21 (46)	28.41 ± 0.69 (6)
Glomus mosseae	0.60 ± 0.1 (11)	85.71 ± 0.48 (2)	1.04 ± 0.6 (62)	80.60 ± 2.54 (0)	1.96 ± 0.15 (40)	33.27 ± 1.61 (24)
Mixture of above three spp. of *Glomus*	0.57 ± 0.06 (6)	87.24 ± 0.76 (4)	0.98 ± .07 (53)	81.67 ± 0.93 (1)	2.01 ± 0.11 (44)	29.18 ± 0.35 (9)
LSD P=0.05	0.15	1.56	0.17	3.52	1.02	5.76
P=0.01	0.17	1.80	0.20	4.07	1.19	6.67

*Main constituent of the essential oil, mean ± standard deviation; * values within parentheses represent per cent increase over control.

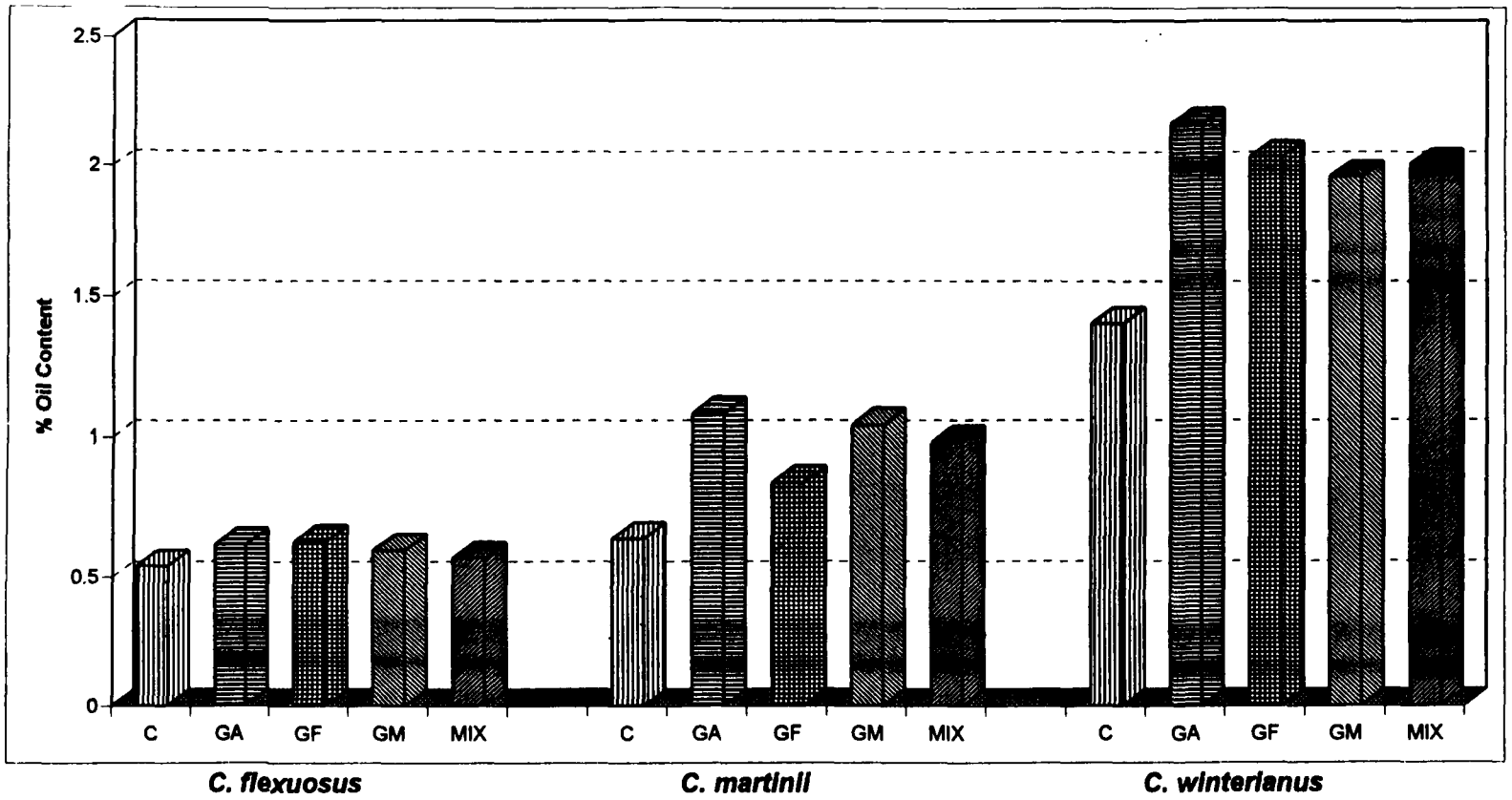


Figure 7. Effect of three different species of *Glomus* on essential oil content of *Cymbopogon* spp. C = Control, GA = *Glomus aggregatum*, GF = *G. fasciculatum*, GM = *G.mosseae*, M = mixture of three *Glomus* spp.

highest increase (69 %) in essential oil content was recorded in plants inoculated with *G. aggregatum* (Fig. 7). Among the VAM fungi tested *G. fasciculatum* caused least effect, the increase being 31 %. Geraniol content in palmarosa plants also did not show significant increase. The geraniol content of the plants ranged between 80.5 to 83.6 % regardless of the species of VAM fungi used for inoculation (Table 6).

***Cymbopogon winterianus* (citronella)**

The inoculation of VAM fungi significantly increased the essential oil content of citronella plants (Fig. 7). The increase in essential oil content was highest (55%) in plants inoculated with *G. aggregatum*. Inoculation of *G. mosseae* brought about the least increase, i.e., only about 40% in essential oil content over that in control plants. However, inoculated citronella plants did not show significant increase in citronellal content of the essential oil. The citronellal content ranged between 26.7 to 33.3 % in citronella oil (Table 6).

Impact of VAM fungi on nutrient uptake and accumulation

Inoculation of the *Cymbopogon* spp. by VAM species (i.e., inoculations by *Glomus aggregatum, G. fasciculatum, G. mosseae* individually and inoculation by their mixture) enhanced the nutrient uptake from the soil (Fig. 8-10, Table 7-11) and their accumulation in plant shoots (Fig. 11-13, Table 12-16).

Influence on nutrient (N,P,K,Cu,Zn) uptake from soil

Nitrogen (N) uptake

VAM inoculation positively influenced the nitorgen uptake by *C.*

flexuosus. Maximum amount of N uptake was observed in *Glomus aggregatum* inoculated plants (28.3 µg/g) which was 25% more as compared to its control. Response of the plants treated with *G. fasciculatum* and mixture of the species was also found to be better as they showed 22% and 20% increase in N uptake over their respective control plants. Response of the plants inoculated with *G. mosseae* with respect to N uptake was lowest. It was only 25.7 µg/g which was 13% more than control plants (Table 7, Fig. 8).

Nitorgen uptake in *C. martinii* plants treated with different VAM spp. was also better than their uninoculated (control) plants. VAM inoculation brought about significant increase in N uptake in all the treatments which ranged from 29 to 40%. Effect of inoculation of *G.aggregatum* and *G. fasciculatum* was similar i.e, 40% increase in N uptake from soil as compared to control plants. Plants inoculated with *G. mosseae* showed lowest N uptake and increase was only 29%. Single inoculation of *G. aggregatum* and *G. fasciculatum* was more effective in influencing the N uptake than their mixed inoculation as it caused 35% increase in the uptake (Table 7, Fig. 9).

Impact of VAM inoculation on N uptake by *C. winterianus* was found to be better than the other two *Cymbopogon* spp. All the four treatments of *C. winterianus* brought about significant increase in N uptake which ranged from 53 to 67% over the controls. Inoculation with *G. mosseae* was least effective as it brought about 53% increase in N uptake. Other three treatments caused significantly greater increase in N uptake by *C. winterianus* plants than by the treatment with *G. mosseae* (Table 7, Fig. 10).

Table 7. Effect of VAM inoculation on uptake of nitrogen from the soil of some *Cymbopogon* spp.

Treatments	Uptake of nitrogen (µg/g soil)		
	C. flexuosus	*C. martinii*	*C. winterianus*
Control	22.7± 0.6	22.7 ± 0.6	19.3 ± 0.6
Glomus aggregatum	28.3 ± 0.6 (25)*	31.7 ± 0.6 (40)	32.3 ± 0.6 (67)
Glomus fasciculatum	27.7 ± 0.6 (22)	31.7 ± 0.6 (40)	32.0 ± 1.0 (66)
Glomus mosseae	25.7 ± 0.6 (13)	29.3 ± 0.6 (29)	29.6 ± 0.6 (53)
Mixture of the above three spp. of *Glomus*	27.3 ± 0.6 (20)	30.7 ± 0.6 (35)	32.0 ± 1.0 (66)
LSD P=0.05	1.19	1.1	1.5
P=0.01	1.75	1.7	2.2

*Figures in parentheses are per cent increase over their respective controls.

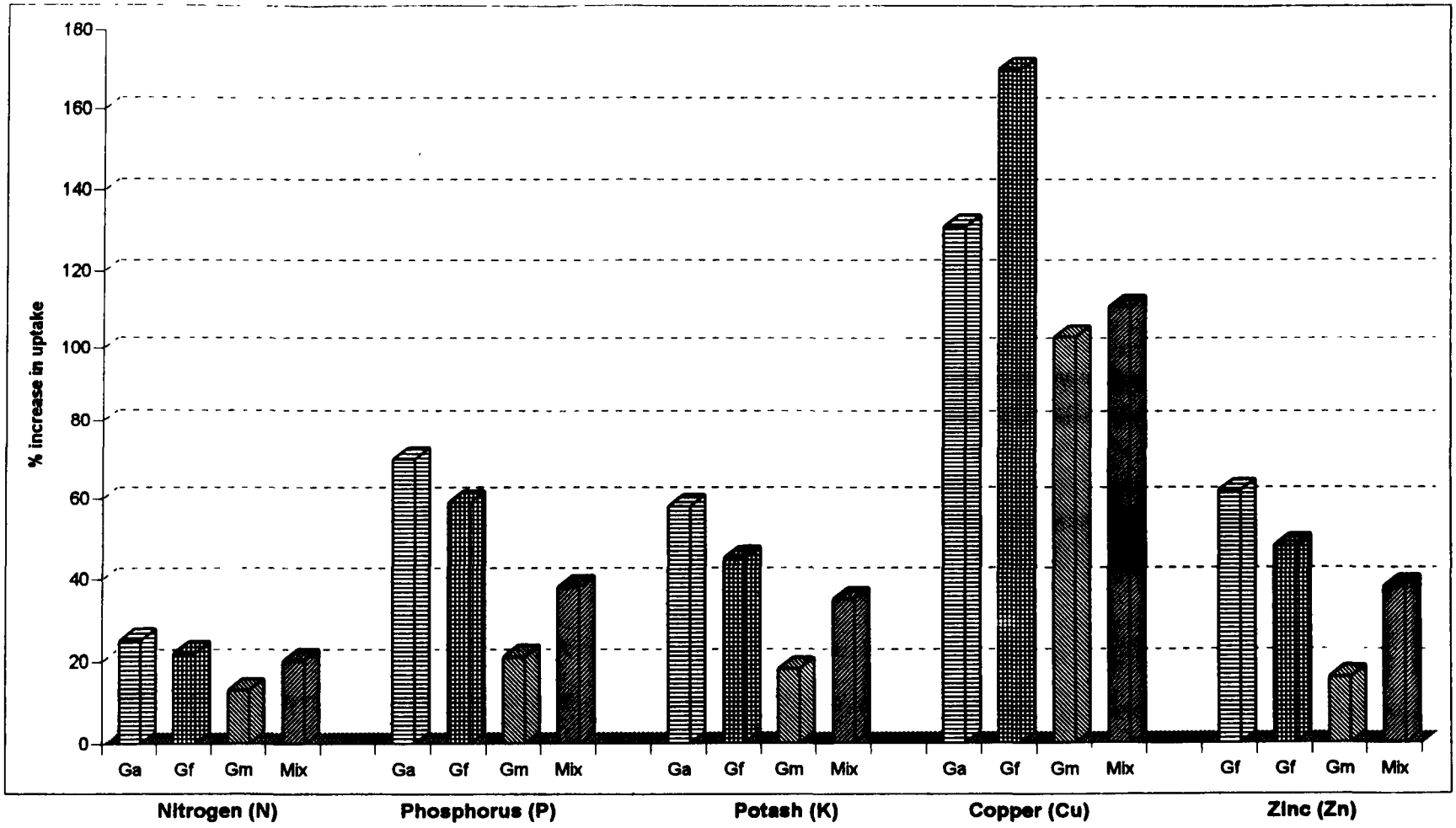


Figure 8. Percent increase in uptake of some macro-and micro nutrients from soil in VAM inoculated *Cymbopogon flexuosus.* Ga=*Glomus aggregatum*, Gf=*G.fasciculatum*, Gm=*G.mosseae*, Mix=mixture of three *Glomus* spp.

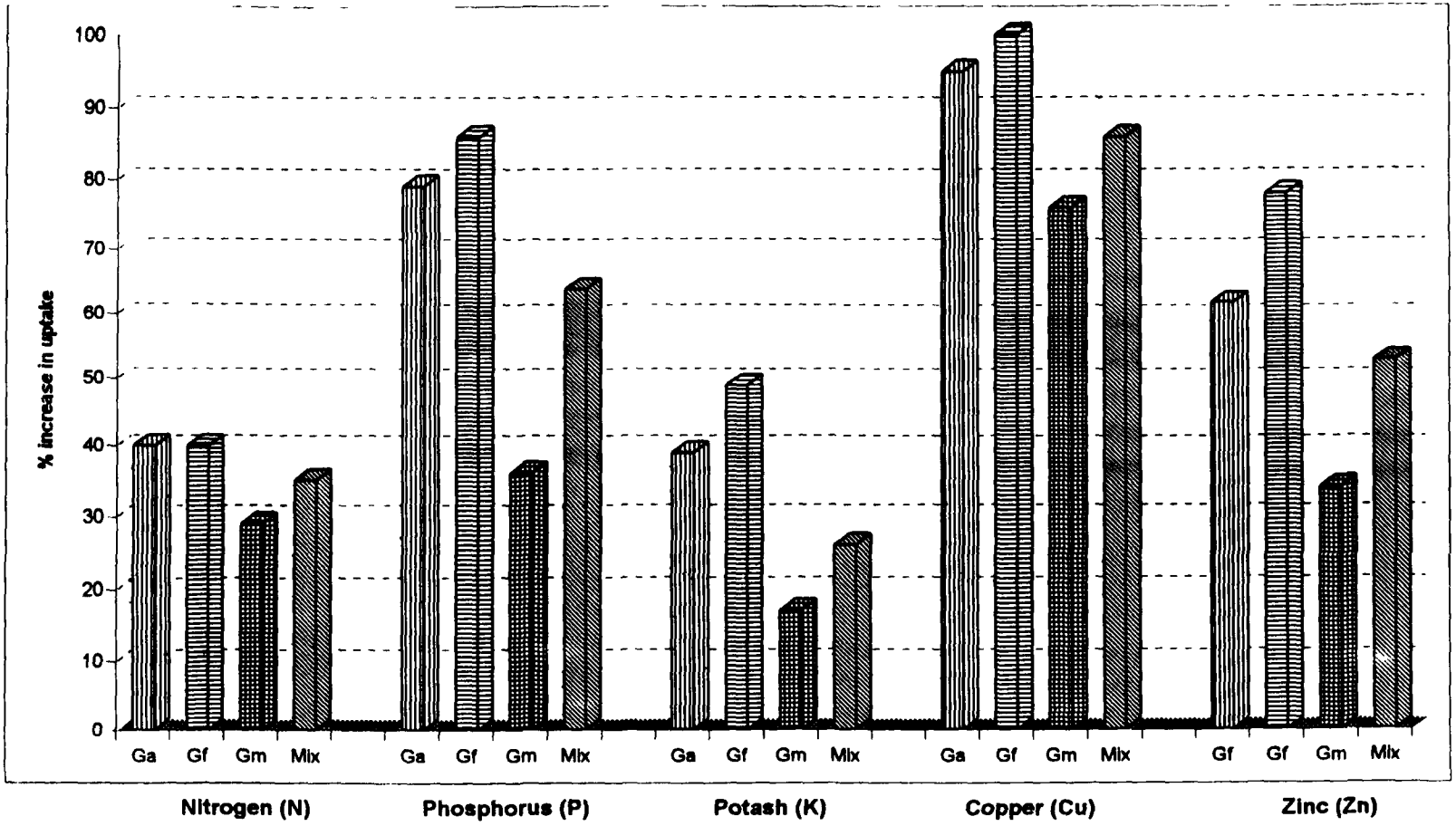


Figure 9. Per cent increase in uptake of some macro- and micro nutrients from soil in VAM inoculated *Cymbopogon martinii*. Ga = *Glomus aggregatum*, Gf =*G. fasciculatum*, Gm=*G. mosseae*, Mix =mixture of three *Glomus* spp.

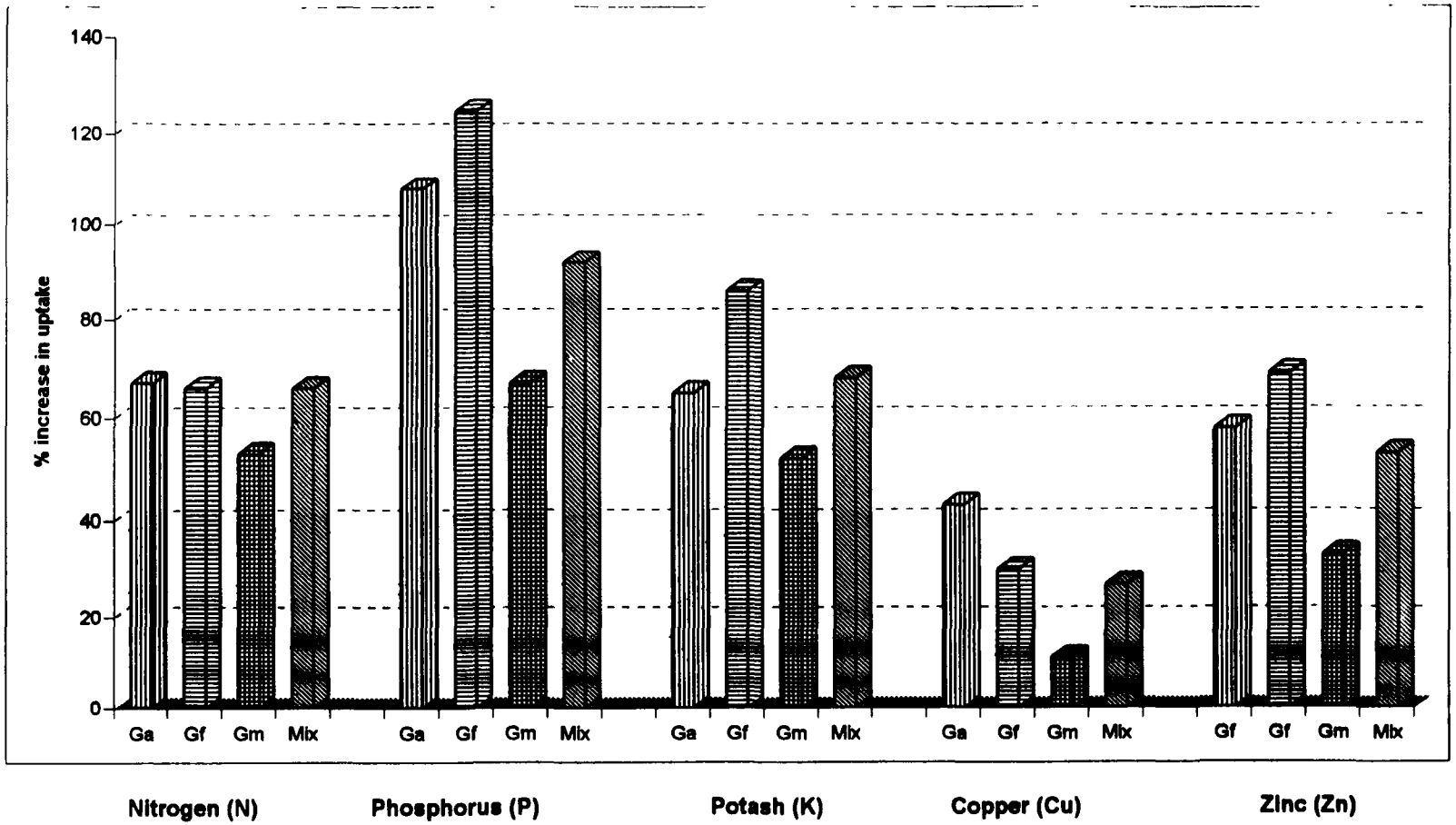


Figure 10. Per cent increase in uptake of some macro- and micro nutrients from soil in VAM inoculated *Cymbopogon winterianus*. Ga = *Glomus aggregatum*, Gf = *G. fasciculatum*, Gm = *G. mosseae*, Mix = mixture of three *Glomus* spp.

Phosphorus (P) uptake

Like nitrogen, VAM inoculation influenced the P uptake by *C. flexuosus* plants. The over all impact of inoculation on P uptake was found to be better than the impact on N uptake (Fig. 8). A significant increase in P uptake was observed in VAM inoculated plants in comparison to their control plants (Table 8). *C. flexuosus* plants incoulated with *G. aggregatum* showed maximum amount of P uptake from soil i.e., 2.43 µg/g which was the highest enhancement (70%) among the treatments. *G. mosseae* caused least effect on P uptake as it increased only 21% P uptake by the plants. Inoculation by *G. fasciculatum* and the mixture brought about 59 and 38% increase in P uptake, respectively (Table 8, Fig. 8).

VAM fungi also increased P uptake by *C. martinii.* P uptake by *C. martinii* due to inoculation with VAM fungi was greater as compared to N uptake by the plants (Fig. 9). *G. fasciculatum* caused highest increase in P uptake from soil. The increase was 86% over control (uninoculated) plants. The effect of mixed inoculation on P uptake also showed the same trend, i.e, lower than the effect of single inoculation of *G. aggregatum G. fasciculatum* and better than *G. mosseae* (Table 8, Fig. 9).

VAM inoculation influenced the P uptake by the three *Cymbopogon* spp. The impact on P uptake by *C. winterianus* plants was greatest among three *Cymbopogon* spp. included in the study. Significant increase occurred in P uptake by all the treatments, the maximum being 125% by *G. fasiculatum* in comparison to control plants. Inoculation by *G. aggregatum* and the

Table 8. Effect of VAM inoculation on uptake of phosphorus from the soil of some *Cymbopogon* spp.

Treatments	Uptake of phosphorus (µg/g soil)		
	C. flexuosus	*C. martinii*	*C. winterianus*
Control	1.43± 0.06	1.4 ± 0.1	1.2 ± 0.1
Glomus aggregatum	2.43 ± 0.06 (70)*	2.5 ± 0.1 (79)	2.5 ± 0.1 (108)
Glomus fasciculatum	2.27 ± 0.06 (59)	2.6 ± 0.1 (86)	2.7 ± 0.1 (125)
Glomus mosseae	1.73 ± 0.06 (21)	1.9 ± 1.6 (36)	2.0 ± 0.1 (67)
Mixture of the above three spp. of *Glomus*	1.97 ± 0.06 (38)	2.3 ± 0.1 (64)	2.3 ± 0.1 (92)
LSD P=0.05	0.12	0.1	0.2
P=0.01	0.17	0.2	0.3

*Figures in parentheses are per cent increase over their respective controls.

mixture of the VAM fungi also increased P uptake by 108% and 92%, respectively. The increase by inoculation with *G. mosseae* was, however 67% in comparison to control plants. This was lowest among all the treatments (Table 8, Fig. 10).

Potassium (K) uptake

All the *C. flexuosus* plants inoculated with the VAM fungi showed marked enhancement in K uptake. The trend in enhancement of K uptake was similar to the trend obseverd in case of N and P uptake (Fig. 8). Highest increase (58%) in the uptake occurred with inoculatation by *G. aggregatum*. *G. mosseae* effected lowest (18%) increase. Effect of inoculation with the mixture of VAM fungi was lower than inoculation with *G. aggregatum* and *G. fasciculatum* alone. Effect of the VAM inoculation on K uptake by *C. flexuosus* plants in gerneral, was better than the effect on N uptake. But the effect was lower than P uptake (Table 9, Fig. 8).

VAM inoculated *C. martinii* plants also showed significant increase in their K uptake which ranged between 17 and 49% compared to control plants. Different treatments with VAM on K uptake followed the same trend as observed in case of P uptake. The effect of different treatments on K uptake was, however, less than P uptake. The enhancement in K uptake by *C. martinii* due to VAM application was also found to be less than that of the *C. flexuosus* plants (Table 9, Fig. 9).

Increase pattern in K uptake by *C. winterianus* plants inoculated with

Table 9. Effect of VAM inoculation on uptake of potassium from the soil of some *Cymbopogon* spp.

Treatments	Uptake of potassium (µg/g soil)		
	C. flexuosus	*C. martinii*	*C. winterianus*
Control	16.3 ± 0.6	17.7 ± 0.6	14.7 ± 0.6
Glomus aggregatum	25.7 ± 0.6 (58)*	24.7 ± 0.6 (39)	24.3 ± 0.6 (65)
Glomus fasciculatum	23.7 ± 0.6 (45)	26.3 ± 0.6 (49)	27.3± 0.6 (86)
Glomus mosseae	19.3 ± 0.6 (18)	20.7 ± 0.6 (17)	22.3 ± 0.6 (52)
Mixture of the above three spp. of *Glomus*	22.0 ± 1.0 (35)	22.3 ± 0.6 (26)	24.7 ± 0.6 (68)
LSD P=0.05	1.2	1.1	1.2
P=0.01	1.8	1.6	1.7

*Figures in parentheses are per cent increase over their respective controls.

VAM fungi was similar to that observed for P uptake (Fig.10). Inoculated *C. winterianus* plants showed better K uptake than the other two *Cymbopogon* spp. The enhancement in K uptake ranged from 52 to 86% over control plants (Table 9, Fig. 10).

Copper (Cu) uptake

Uptake of Cu was also enhanced by inoculation of *Cymbopogon* spp. with VAM fungi. The trend of Cu uptake in *C. flexuosus* plants was similar to those as observed for NPK. Copper uptake was highest (83 μg/g) by *G. aggregatum* inoculated plants which was 131% greater than control plants. Plants incoulated with *G. mosseae* showed less absorption and the increase in the uptake was 103%. *C. flexuosus* plants inoculated with VAM spp. showed better Cu uptake than other two *Cymbopogon* spp (Table 10, Fig. 8).

C. martinii plants inoculated with different VAM spp. showed significantly increased Cu uptake. The enhancement in the Cu uptake by the inoculated *C. martinii* plants ranged between 76 and 100% compared to control plants (Table 10, Fig. 9).

Enchancement of Cu uptake by *C. winterianus* plants inoculated with VAM fungi was lowest in comparison to other *Cymbopogon* spp. The amount of Cu uptake ranged from 0.41 to 0.53 μg/g in comparison to 0.37 μg/g uptake by control plants. The increase in Cu uptake was higest (43%) by *G. aggregatum* treated plants where as lowest (11%) by *G. mosseae* in comparison to control plants (Table 10, Fig. 10).

Table 10. Effect of VAM inoculation on uptake of copper from the soil of some *Cymbopogon* spp.

Treatments	Uptake of copper (µg/g soil)		
	C. flexuosus	*C. martinii*	*C. winterianus*
Control	0.36± 0.01	0.37 ± 0.01	0.37 ± 0.01
Glomus aggregatum	0.83 ± 0.01 (131)*	0.72 ± 0.01 (95)	0.53 ± 0.01 (43)
Glomus fasciculatum	0.78 ± 0.01 (117)	0.74 ± 0.01 (100)	0.48 ± 0.01 (30)
Glomus mosseae	0.73 ± 0.01 (103)	0.65± 0.01 (76)	0.41 ± 0.01 (11)
Mixture of the above three spp. of *Glomus*	0.76± 0.01 (111)	0.69 ± 0.01 (86)	0.47± 0.01 (27)
LSD P=0.05	0.01	0.01	0.01
P=0.01	0.02	0.02	0.02

*Figures in parentheses are per cent increase over their respective controls.

Zinc (Zn) uptake

Uptake of Zn was increased by inoculation of *Cymbopogom* spp. with VAM fungi. *C. flexusous* plants treated with *G. aggregatum* showed highest uptake of Zn (1.02 μg/g) which was 62% more than its uninoculated control and those treated with *G. mosseae* responded least as only 16% increase was observed (Table 11).

C. matrinii plants treated with VAM spp. showed the same trend as in case of P,K and Cu uptake (Fig. 9). Increase in Zn uptake brought about by *C. martinii* plants inoculated with VAM fungi was observed to be highest among three *Cymbopogon* spp. studied and it ranged from 34 to 78%, compared to control plants (Table 11, Fig. 9).

A positive effect on Zn uptake occurred by inoculation of *C. winterianus* plants with VAM fungi. The Zn uptake was significantly increased by all the treatments and varied from 33 to 69%. The maximum enchancement was brought about by *G. fasiculatum* and least by *G. mosseae* treated plants (Table 11, Fig. 10).

Influence on nutrient accumulation in plant shoots

Nitrogen (N) accumulation

Inoculation of *C. flexuosus* plants with the VAM fungi markedly influenced the accumulation of N in plant tissues. Increase in accumulation of N was upto 92% in the plants inoculated with *G. aggregatum*. Considerable

Table 11. Effect of VAM inoculation on uptake of Zinc from the soil of some *Cymbopogon* spp.

Treatments	Uptake of zinc (µg/g soil)		
	C. flexuosus	*C. martinii*	*C. winterianus*
Control	0.63 ± 0.01	0.64 ± 0.01	0.64 ± 0.01
Glomus aggregatum	1.03 ± 0.01 (62)*	1.04 ± 0.01 (62)	1.01 ± 0.00 (58)
Glomus fasciculatum	0.93 ± 0.01 (48)	1.14 ± 0.01 (78)	1.08 ± 0.01 (69)
Glomus mosseae	0.73 ± 0.01 (16)	0.86 ± 0.01 (34)	0.85 ± 0.01 (33)
Mixture of the above three spp. of *Glomus*	0.87 ± 0.01 (38)	0.98 ± 0.01 (53)	0.98 ± 0.01 (53)
LSD P=0.05	0.010	0.01	0.01
P=0.01	0.015	0.02	0.015

*Figures in parentheses are per cent increase over their respective controls.

increase was also found in plants inoculated with *G. fasciculatum* (75%). Lowest increase (27%) occured in plants inoculated with *G. mosseae* (Table 12, Fig. 11).

In *C. martinii* plants the effect of *G. fasciculatum* was greatest as it brought about 99% increase in comparison to control plants. Inoculation with *G. aggregatum* and with the mixture of the VAM fungi caused 80 and 66% increase in N accumulation in shoots, respectively. *G. mosseae* treated plants showed least effect i.e., 26% increase (Table 12, Fig. 12).

Enhancement in N accumulation by inoculation with *C. winterianus* plants varied between 31 to 90% over control plants. Maximum amount (24.7 mg/g tissues) of N was found accumulated in *G. fasiculatum* inoculated plants which was 90% more than control plants. Inoculation of *G. aggregatum* and mixture of the VAM fungi also enhanced N accumulation which were 67 and 46% respectively more than control plants. *G. mosseae* inoculated plants showed only 31% increase (Table 12, Fig.13).

Phosphorus (P) accumulation

Inoculation with the VAM fungi enchanced P accumulation in the shoot tissue of *Cymbopogon* spp. Phosphorus accumulation was greater than any other nutrients. As a result of inoculation of *C. flexuosus* plants with VAM fungi P accumulation increased. This increase was upto 100% over control plants by the inoculation with *G. aggregatum*. Inoculation with *G. fasciculatum* (78%) and mixture of the VAM fungi (61%) also brought about increased P

Table 12. Effect of VAM inoculation on accumulation of nitorgen in shoot tissues of some *Cymbopogon* spp.

Treatments	Accumulation of nitrogen (mg/g tissue)		
	C. flexuosus	*C. martinii*	*C. winterianus*
Control	12.0 ± 1.0	13.7 ± 0.6	13.0 ± 1.0
Glomus aggregatum	23.0 ± 1.0 (92)*	24.7 ± 0.6 (80)	21.7 ± 0.6 (67)
Glomus fasciculatum	21.0 ± 0.6 (75)	27.3 ± 0.6 (99)	24.7 ± 0.6 (90)
Glomus mosseae	15.3 ± 0.6 (27)	17.3 ± 0.6 (26)	17.0 ± 1.0 (31)
Mixture of the above three spp. of *Glomus*	18.3 ± 0.6 (52)	22.7 ± 0.6 (66)	19.0 ± 1.0 (46)
LSD P=0.05	1.8	0.8	1.8
P=0.01	2.6	1.2	2.6

*Figures in parentheses are per cent increase over their respective controls.

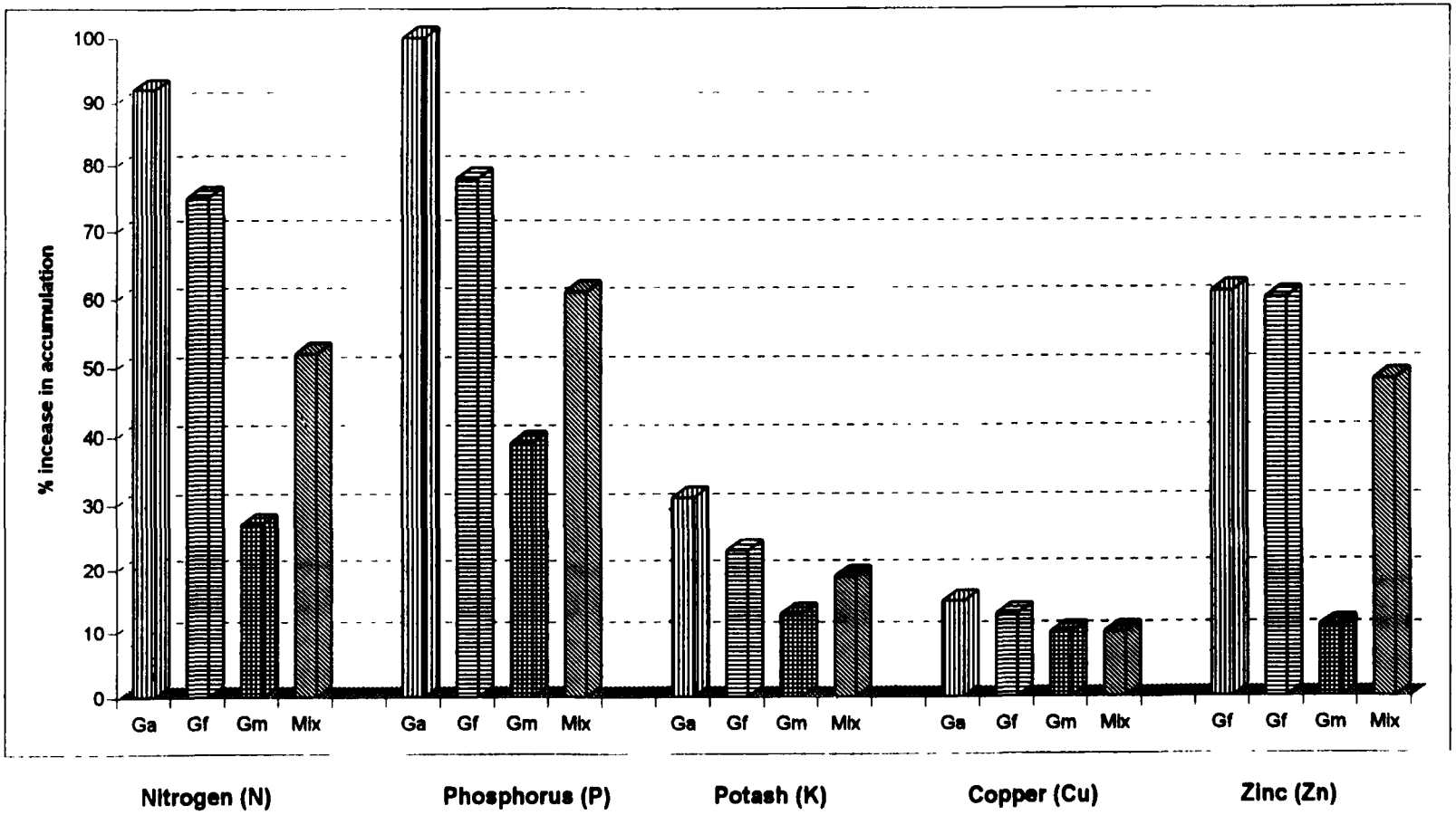


Figure 11. Per cent increase in accumulation of some macro- and micro nutrients in shoots of VAM inoculated *Cymbopogon flexuosus*. Ga = *Glomus aggregatum*, Gf = *G. fasciculatum*, Gm = *G. mosseae*, Mix = mixture of three *Glomus* spp.

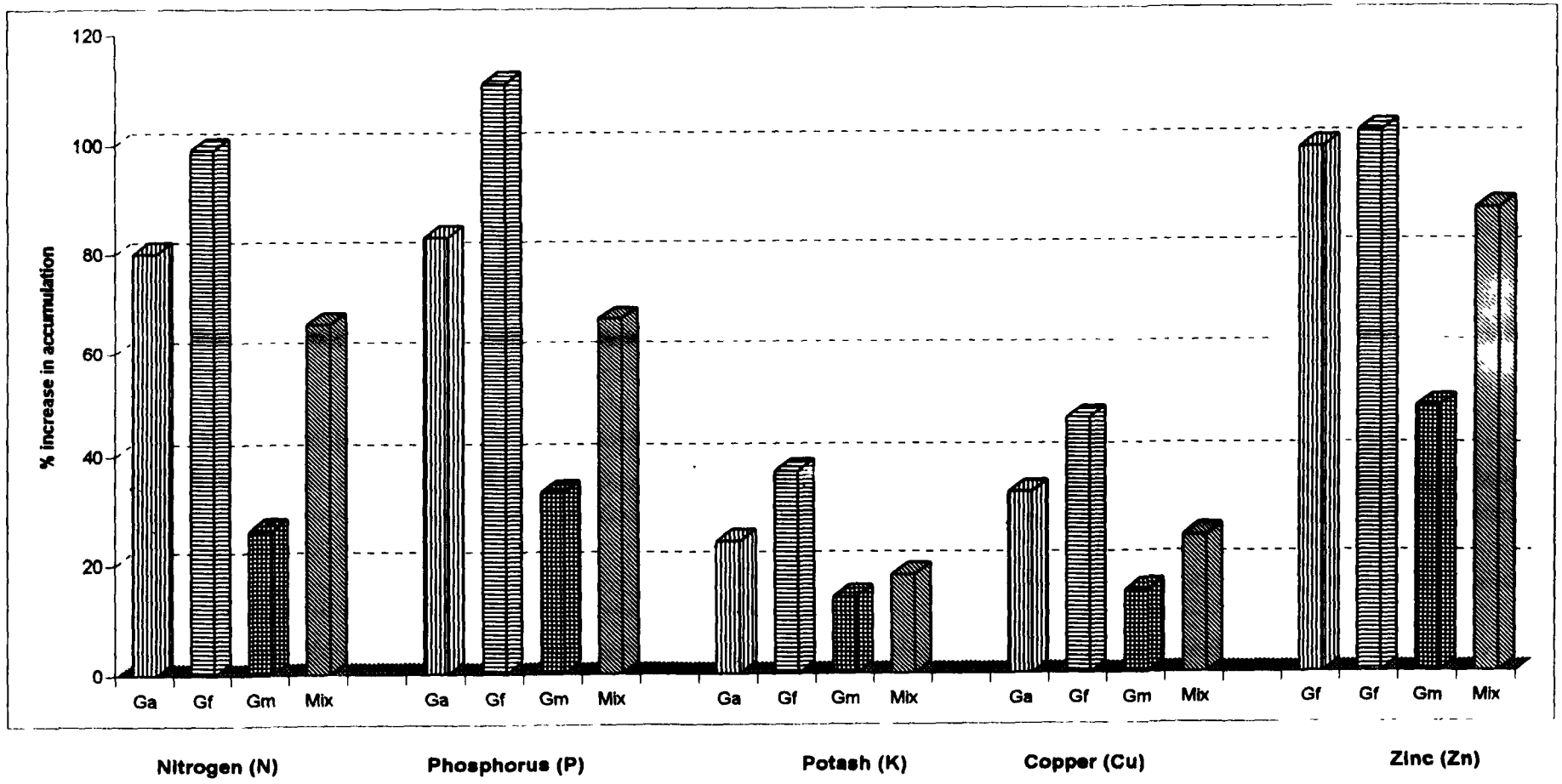


Figure 12. Per cent increase in accumulation of some macro- and micro nutrients in shoots of VAM inoculated *Cymbopogon martinii* . Ga = *Glomus aggregatum*, Gf = *G. fasciculatum* , Gm = *G. mosseae* , Mix = mixture of three *Glomus* spp.

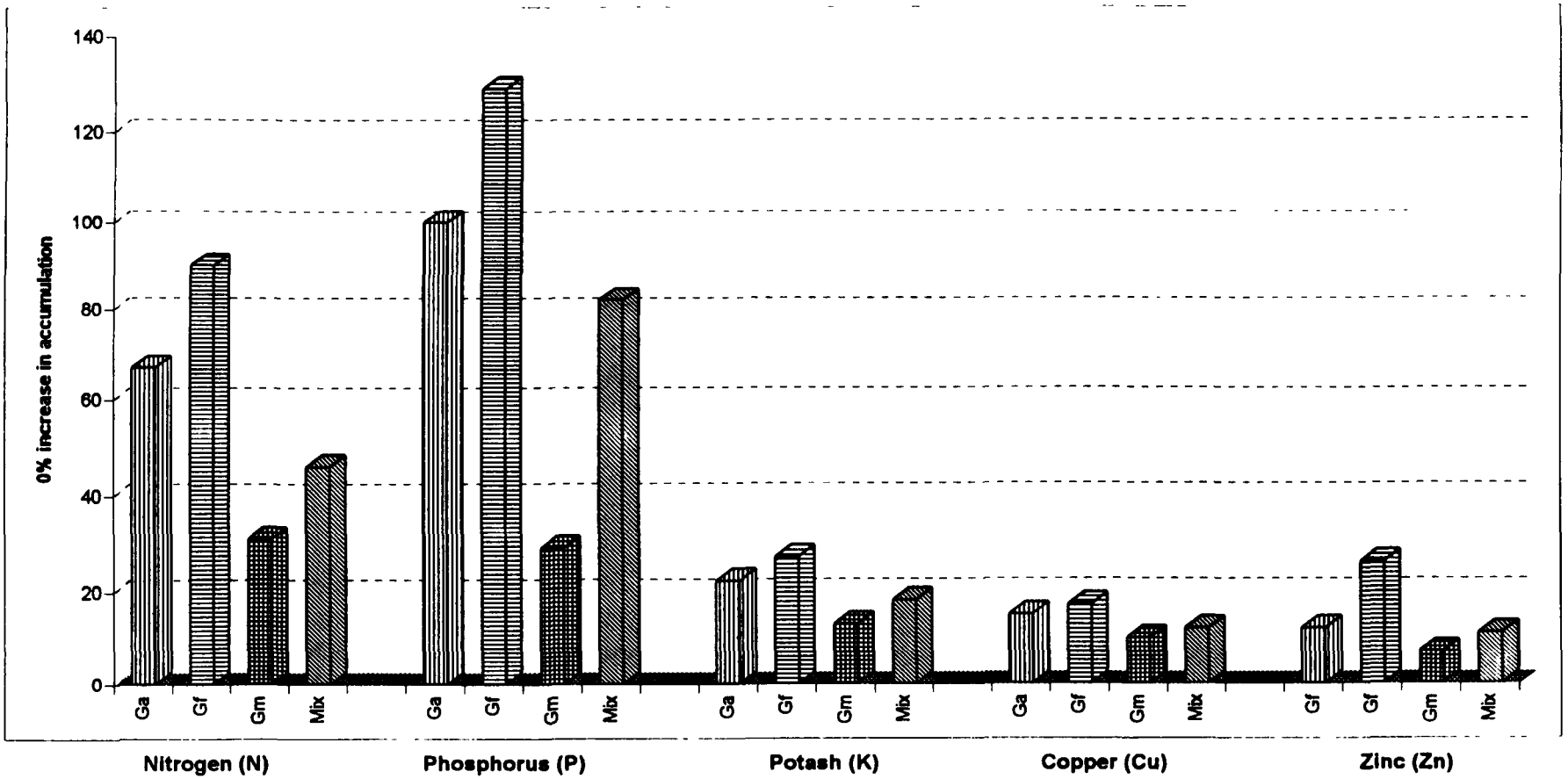


Figure 13. Per cent increase in accumulation of some macro- and micro nutrients in shoots of VAM inoculated *Cymbopogon winterianus*. Ga = *Glomus aggregatum*, Gf = *G. fasciculatum*, Gm = *G. mosseae*, Mix = mixture of three *Glomus* spp.

accumulation. The increase due to *G. mosseae* inoculation was least effective in this respect as the increase was only 39% (Table 13, Fig. 11).

Plants of *C. martinii* inoculated with the VAM fungi also showed increase in P accmulation in plant shoots. It ranged between 33 and 111% in comparison to control plants. Maximum increase was observed by inoculation with *G. fasciculatum*, whereas minimum accumulation was found by inoculation with *G. mosseae* (Table 13, Fig. 12).

Cymbopogon winterianus plants inoculated with different VAM spp. also showed enhanced P accumulation. The enhancement ranged between 29 and 129% in comparison to control plants. Maximum enchancement was brought about by *G. fasciculatum* and minimum by *G. mosseae*. Considerable increase in P accumulation was recorded in plants inoculated with *G. aggregatum* (100%) and mixture of the species to about 82% (Table 13, Fig. 13).

Potassium (K) accumulation

Inoculation with different VAM fungi influenced K accumulation in shoots of *C. flexuosus* plants to some extent. The trend in K accumulation was similar to N and P accumulation in shoot tissues but the extent of enhancement in accumulation was comparatively low. It ranged between 13 and 31% compared to control plants (Table 14, Fig 11).

Cymbopogon martinii plants inoculated with the VAM fungi ahowed

Table 13. Effect of VAM inoculation on accumulation of phosphorus in shoot tissues of some *Cymbopogon* spp.

Treatments	Accumulation of phosphorus (mg/g tissue)		
	C. flexuosus	*C. martinii*	*C. winterianus*
Control	1.8 ± 0.1	1.8 ± 0.6	1.7 ± 0.1
Glomus aggregatum	3.6 ± 0.1 (100)*	3.3 ± 0.6 (83)	3.4 ± 0.1 (100)
Glomus fasiculatum	3.2 ± 0.1 (78)	3.8 ± 0.6 (111)	3.9 ± 0.1 (129)
Glomus mosseae	2.5 ± 0.1 (39)	2.4 ± 0.1 (33)	2.2 ± 0.1 (29)
Mixture of the above three spp. of *Glomus*	2.9 ± 0.1 (61)	3.0 ± 0.6 (67)	3.1 ± 0.1 (82)
LSD P=0.05	0.1	0.09	0.14
P=0.01	0.2	0.13	0.20

*Figures in parentheses are per cent increase over their respective controls.

increased K accumulation. The trend was similar as in N and P accumulation. The increase was found to be less than N and P accumulation. The highest increase in K accumulations (37%) was brought about by inoculation with *G. fasciculatum* and minimum (14%) with *G. mosseae* (Table 14, Fig. 12).

Highest increase in K accumulation in *C. winterianus* plants was observed in *G. fasciculatum* inoculated plants. The enchancement in accumulation was 27% in comparison to the control plants. Plants inoculated with *G. aggregatum* also showed better K accumulation (22% increase). *G. mosseae* and the mixture of the VAM fungi caused only 13 and 18% increase respectively (Table 14, Fig. 13).

Copper (Cu) accumulation

VAM fungi also enchanced Cu accumulation in the plant shoots of *Cymbopogon* spp. Inoculation of *C. flexuosus* plants with the VAM significantly influenced Cu accumulation in shoot tissues. The increase ranged from 10 to 15%, compared to the control plants but the effect of different treatments did not differ significantly (Table 15, Fig. 11).

C. martinii plants also showed an increase in Cu accumulation which ranged from 15 to 47% in comparison to control plants. VAM inoculated *C. martinii* plants showed better Cu accumulation than other two *Cymbopogon* spp. Highest increase in Cu accumulation occurred due to inoculation with *G. fasciculatum.* Plants inoculated with *G. aggregatum* also showed 33% increased Cu accumulation in comparison to control plants (Table 15.,

Table 14. Effect of VAM inoculation on accumulation of potassium in shoot tissues of some *Cymbopogon* spp.

Treatments	Accumulation of potassium (mg/g tissue)		
	C. flexuosus	*C. martinii*	*C. winterianus*
Control	14.0 ± 0.1	14.2 ± 0.6	13.8 ± 0.1
Glomus aggregatum	18.4 ± 0.1 (31)*	17.6 ± 0.6 (24)	16.9 ± 0.1 (22)
Glomus fasciculatum	17.2 ± 0.1 (23)	19.5 ± 0.1 (37)	17.6 ± 0.1 (27)
Glomus mosseae	15.8 ± 0.0 (13)	16.2 ± 0.6 (14)	15.6 ± 0.1 (13)
Mixture of the above three spp. of *Glomus*	16.6 ± 0.1 (19)	16.8 ± 0.6 (18)	16.3 ± 0.1 (18)
LSD P=0.05	0.2	0.1	0.2
P=0.01	0.3	0.2	0.3

*Figures in parentheses are per cent increase over their respective controls.

Table 15. Effect of VAM inoculation on accumulation of copper in shoot tissues of some *Cymbopogon* spp.

Treatments	Accumulation of copper (µg/g tissue)		
	C. flexuosus	*C. martinii*	*C. winterianus*
Control	15.7 ± 0.6	19.7 ± 0.6	15.7 ± 0.6
Glomus aggregatum	18.0 ± 0.0 (15)*	26.3 ± 0.6 (33)	18.0 ± 0.0 (15)
Glomus fasciculatum	17.7 ± 0.6 (13)	29.0 ± 1.0 (47)	18.3 ± 0.6 (17)
Glomus mosseae	17.3 ± 0.6 (10)	22.7 ± 0.6 (15)	17.3 ± 0.6 (10)
Mixture of the above three spp. of *Glomus*	17.3 ± 0.6 (10)	24.7 ± 0.6 (25)	17.6 ± 0.6 (12)
LSD P=0.05	1.0	1.3	0.9
P=0.01	1.5	1.9	1.3

*Figures in parentheses are per cent increase over their respective controls.

Fig.12).

Significant increase in Cu accumulation also occurred in *C. winterianus* plants due to the inoculations. The increase varied from 10 to 17%. But the enhancement in Cu accumulation due to different treatments did not differ significantly (Table 15, Fig. 13).

Zinc (Zn) accumulation

Zinc accumulation was also influenced by VAM fungi. Increase in Zn accumulation in inoculated *C. flexuosus* plant shoots ranged from 11 to 61% in comparison to control plants. *G. aggregatum* and *G. fasciculatum* inoculated plants also showed increased Zn accumulation which was 60 and 61%, respectively. Lowest increase was effected by *G. mosseae* (Table.16, Fig. 11).

Effect of VAM inoculation on Zn accumulation was observed to be best in *C. martinii* plants. The increase of 102% was brought about by the inoculation of *G. aggregatum*. Considerable increase in Zn accumulation was also occurred by the inoculation with *G. fasciculatum* (99%) and mixture of VAM spp. (88%). Least effect was observed by *G. mosseae* inoculated plants showed least enhanced accumulation, i.e., 49% (Table 16, Fig. 12).

An increase in Zn accumulation in *C. winterianus* plants also occurred. Highest increase was brought about by *G. fasciculatum* which was 26% greater than control plants. Plants inoculated with *G. aggregatum* and mixture of the species showed as 12 and 11% increase, respectively. *G. mosseae*

Table 16. Effect of VAM inoculation on accumulation of zinc in shoot tissues of some *Cymbopogon* spp.

Treatments	Accumulation of zinc (µg/g tissue)		
	C. flexuosus	*C. martinii*	*C. winterianus*
Control	33.3 ± 0.6	31 ± 1.0	30.0 ± 1.0
Glomus aggregatum	53.7 ± 0.6 (61)*	61.7 ± 0.6 (99)	33.7 ± 0.6 (12)
Glomus fasciculatum	53.3 ± 0.6 (60)	62.7 ± 0.6 (102)	37.7 ± 0.6 (26)
Glomus mosseae	37.0 ± 1.0 (11)	46.3 ± 0.6 (49)	32.0 ± 0.0 (7)
Mixture of the above three spp. of *Glomus*	49.3 ± 0.6 (48)	58.3 ± 0.6 (88)	33.3 ± 0.6 (11)
LSD P=0.05	1.4	1.2	1.2
P=0.0 1	2.1	1.8	1.8

*Figures in parentheses are per cent increase over their respective controls.

caused only 7% increase in Zn accumulation (Table 16, Fig. 13).

DISCUSSION

The cultivated *Cymbopogon* spp. are colonized by a number of VAM fungi (Barthakur and Bordoloi, 1990). In the present investigation. all the five selected *Cymbopogon* spp. (*C. caesius, C. flexuosus, C. martinii, C. pendulus* and *C. winterianus*) showed VAM associations and are therefore, largely VAM dependent in nature. The degree of VAM colonization, however, varied. The colonization was mostly characterized by the extensive development of vesicles and intramatrical spores in the root cortex. The reduction in number of arbuscules as compared to vesicles in the VAM colonized roots appeared to be caused by the loss of fine roots. Saif and Khan (1975) reported that in wheat arbuscule formation was prominent during early stage to the peak of vegetative growth and vesicles were more abundant in older root tissues. The fine roots often deteriorated after flowering, seed-setting or after the harvest of tops. A significant reduction in number of arbuscules due to abundant older roots in *Mentha* spp. has also been reported (Abdul-Khaliq, 1993). It is well known that VAM fungi usually occur in root cortical cells. The VAM fungi associated with palmarosa roots have been found to occur in the tissues inside vascular cylinder including large metaxylem and pith cells. The reports on the occurrence of VAM fungi in vascular tissue are meagre. The vascular tissue of ginger have earlier been reported to show the presence of VAM fungi (Taber and Trappe, 1982). The transverse section of palmarosa roots revealed that the endodermal cells are thin and lack a regular casperian strip. It restricts free passages of nutrients between cortex and vascular cylinder. The actual cause

and mode of entrance of VAM fungi inside the vascular cylinder is, however, not known and required to be investigated for.

No direct correlation was found in the spore counts (density) in the rhizosphere soil of *Cymbopogon* spp. with the intensity of VAM colonization in roots. Several factors might have worked to regulate spore counts. The major factor might be the influence of the host itself on the development of mycorrhizal association. Growth and multiplication of VAM fungi often depends upon the host plants (St. John, 1980), fertilizer application (Ross, 1971; Verkade and Hamilton, 1985; Johnston *et al.*, 1986;) and light intensity (Graham *et al.*, 1982; Son *et al.*, 1988). Stage of plant growth also influences the production of VAM spore in rhizosphere soil. The multiplication of VAM fungi is favourably influenced by these plants. As these grasses are perennial, slow colonizing and more adaptable endophyte (VAM fungus) may provide better benefits. The association of *G.dimorphicum, G. fasiculatum, G. geosporum, G. macrocarpum, G. multicaulis,G. occultum, G. reticulatum* and one *Gigaspora* spp. with the five *Cymbopogon* spp. included in the study is hereby reported for the first time.

Spore density displayed seasonal variations and there was no correlation between mycorrhizal colonization and spore density in soil. Multiplication of VAM fungi was found affected by the weather conditions. All the five *Cymbopogon* spp. showed a similar trend in this respect. The multiplication of the VAM fungi was inhibited in hot and dry weather conditions of summer months (April, May, June) and promoted by higher relative humidity and

moderate temperature regimes. The highest spore count in the rhizosphere of all the *Cymbopogon* spp. was observed in February and March (average temperature 19.8 and 22°C, respectively). Lopez-Sanchez and Honrubia (1992) reported that spore population remained high during autumn, fell to minimum in winter and tended to increase in spring. On the other hand, Vardavakis (1992) observed that greatest number of spores occurred in summer. In the present study, the lowest spore count was found to be in the month of October, and then sharply increased in November, which remained stable during the winter. During October' 92 an unusual kind of heavy down pour (50.3 mm) for four days resulted into water logged conditions, which might have affected the VAM spores adversely. Otherwise generally the lower spore count was observed during summer and rainy seasons.

Root infection by the VAM fungi showed no correlation with weather conditions. The VAM fungi behaved differently in different *Cymbopogon* spp. However, in general root infection was highest in the months of June and July when temperature was high and humidity low. Lowest root infection in different *Cymbopogon* spp. occurred during any time period between February to April. Siguenza *et al.*, (1996) has made similar observation that colonization percentages were higher in summer than in spring. On the contrary Lopez-Sanchez and Honorubia (1992) reported that infection decreased to minimum in summer. But Vardavakis(1992) reported that highest percentage root infection occurred in spring. Like wise, in another study Shamim *et al.*, (1994) observed that root infection was maximum in spring but gradually decreased

in the following seasons, reaching minimum in winter.

Inoculation of the *Cymbopogon* spp. with VAM fungi significantly improved plant height, number of tillers and fresh weight. The noticeable effect of mycorrhization was evident in all the considered vegetative growth parameters in *C. winterianus*. The enhancement in fresh shoot weight (herb yield) of *C .winterianus* was found to be highest on inoculation with *G. fasciculatum*. Like wise, in *C. martinii* highest level of enhancement in fresh shoot weight was brought about by *G. fasciculatum* . Among the three *Cymbopogon* spp., the response of *C. flexuosus* to VAM inoculation was least for growth enhancement. The influence of *G .mosseae* in increasing fresh herb yield in all three species of *Cymbopogon* was relaltively low. Mycorrhization also significantly increased tillering and height of the plants. Aboul- Nasr (1994) had found increase in plant growth parameters and the number of flowers per plant in VAM inoculated *Tagetes erecta* and *Zinnia elegans*. Bonito *et al.*, (1995) worked on horticultural species inoculated with *Glomus intraradix* and found that shoot dry weight of lettuce, flower buds of zinnia, flower diameter of *Gerbera jamesonii* and plant height of *Dieffenbachia maculata* were increased by inoculation.

The suitability of *G. aggregatum* and *G. fasciculatum* for better biomass production in various plants has also been proved (Verkade and Hamilton, 1985; Baas and Lambers, 1988; Gupta and Janardhanan, 1991; Ganesan and Mahadevan, 1994; Verma and Jamaluddin, 1995). Baas and Lambers (1988) found *G. fasciculatum* effective in increasing plant growth

and shoot root ratio of *Plantago major* subsp. *pleiosperma*. *Glomus fasciculatum* and *G. mosseae* successfully promoted plant growth of *Liriodendron tulipifera* under high fertility (Verkade and Hamilton, 1985). Verkade and Hamilton (1987) found significant increase of fresh weight of *Viburnum dentatum* by inoculating the plants with *G. fasciculatum*. Verkade *et al.*, (1988) observed that *G. fasciculatum* was more effective than *G. macrocarpum* in symbiotic activity. Similarly Sainz and Arines (1988) found better effect of *G .fasciculatum* E_3 strain for red clover in comparison to other four VAM fungi. Enhanced plant growth and biomass production in palmarosa (*C. martinii*) by inoculation of the plants with *G. aggregatum* was reported earlier by Gupta and Janardhanan (1991). Verma and Jamaluddin (1995) reported that inoculation of *Tectona grandis* seedlings with *G. fasciculatum* or mixed AM fungi increased plant height and biomass production.

Inoculation of the *Cymbopogon* spp. with the three VAM fungi in combinaiton was not very effective. The effect on plant growth performance was less than the effect of *G. aggregatum* or *G. fasciculatum* alone. On the contrary to this finding, Kooman *et al.*, (1987) reported that four VAM species togeather were equally or more effective in promoting plant growth than a single species. Ganesan and Mahadevan (1994) reported that joint inoculations of *G. mosseae* and *G. aggregatum* gave the highest tuber yield/ plant in cassava, elephant foot yam and taro crops. Mandal and Kaushik (1994) working with *Acacia nilotica* found that combined treatment of *G. mosseae*

and *G. gilmorei* was the most effective.

VAM colonization of *Cymbopogon* spp. varied in respect to plant fungus symbiont combnation. The VAM colonization, however, did not show any direct correlation with spore population in the rhizosphere of the selected *Cymbopogon* spp. *Cymbopogon flexuosus* gave best response in plant growth prameters with *G. aggregatum*, but extent of colonization was comparatively less than *G. fasiculatum*. However, in case of *C. martinii* the degree of growth enhancement and VAM colonization was found to be highest with *G. fasciculatum*. In *C. winterianus*, the level of colonization was highest with *G. aggregatum*. *G. fasciculatum* was most efficient. It was almost equally efficient on all the three selected crops. *G. mosseae* was least effective in enhancing fresh herb yield and root colonization.

VAM spore count and growth performance of the plants was not correlated. *G. aggregatum* multiplied almost at the same level in the rhizosphere of all the three *Cymbopogon* spp. which was estimated to be 22 spores g^{-1} soil.*G fasciculatum* did not multiply so well in the rhizosphere in comparison to other two VAM fungi (7 to 12 spores g^{1}). Its inoculation showed best effect on growth performence in *C. martinii* and *C . winterianus*. In *C. flexuosus*, effect of *G. fasciculatum* was also found considerably good, only next to *G. aggregatum*. This shows that *G. fasciculatum* with even lesser number of spores or inoculum had better efficiency in relation to these *Cymbopogon* spp. Enhancement in plant growth and consequently increased herb yield were exhibited by the plants inoculated with the VAM fungi. This finding is of

economic importance. Yield of *Cymbopogon* spp. specially of *G. winterianus* can be improved by the application of VAM fungi for greater economic utility.

VAM fungi caused significant increase in the essential oil content in all the three *Cymbopogon* spp. This increase in essential oil content was related to the enhanced plant growth in responce to VAM inoculation. The leaves are the major source of essential oil rather than the other plant organs. It was observed that inoculated *Cymbopogon* plants were healthy and had relatively more foliage and less stem part. Abdul-Khaliq (1993) observed that in *Mentha arvensis* plants inoculated with *G. mosseae* , no remarkable increase in essential oil content per unit tissue occurred but there was an increase in the total oil content per plant. The increase in total oil content was found related with increase in fresh shoot weight of the plants.

VAM inoculated citronella and lemon-grass plants showed more pronounced enhancement in leaf growth as compared to stem. The inoculated palmarosa plants were found to have relatively thin tillers as compared with control plants having thicker tillers. Apparently, the VAM inoculation significantly altered the carbon allocation patterns. The larger amounts of carbon were feasibly allocated to the leaf than stem. This change in leaf: stem ratio might have increased the oil content per unit tissue weight. Further investigations are needed to determine the mechanism involved and exact cause of increased oil yield.

Mycorrhization of the plants favourably influenced uptake and accumu-

lation of nutrient elements. All the treatments resulted in increased nutrient uptake as well as their occumulation in plants. Many workers reported similar results of increased uptake or accumulation of nutrients (Espinoza-Victoria *et al.*, 1993; Karagiannidis *et al..*, 1995; Osonubi *et al* ., 1995; Al-karaki and Al-Raddad, 1997). Osonubi *et al.*, (1995) studied the effect of VAM inoculation on nutrient uptake and yield of cassava and observed a significant increase in root uptake of N,P,K but leaf accumulation was not found affected except for the P. The nutrient contents (P, Cu, Zn, Mn,Fe) were found higher in mycorrhizal wheat plants than non mycorrhizal (Al-Karaki and Al-Raddad, 1997).

Among the four treatments, combination of *G. aggregatum* with *C. flexuosus* and of *G . fasciculatum* with *C. martinii* and *C. winterianus* were better than other combinations. *G. aggregatum* also caused considerable increase in *C. martinii* and *C. winterianus* plants. Uptake as well as accumulation of N,P,K, Cu and Zn was found maximum in these treatments. *G. mosseae* with all the three *Cymbopogon* spp. was least effective. The effect of inoculation of any *Cymbopogon* spp. by the mixtures of VAM fungi on nutrient uptake and accumulation was not as much as produced by single inoculation of *G. aggregatum* and *G. fasciculatum* alone. Espinoza-Victoria *et al.*, (1993), when inoculated native corn with *G. etunicatum, G. mosseae* and *G . pallidium* singly and also in combination, observed that plants colonized by mixture of three inocula did not reflect the effects produced by *G. etunicatum* alone. On the contrary, when working with sunflower they

observed markedly better response of mixed inoculation in comparison to single inoculations (Espinoza-Victoria *et al.* 1993).

The plants in general irrespective of the VAM species or the species involved showed greater uptake and accummlation of phosphorus. Several studies earlier have recorded enhanced phosphorus uptake by mycorrhizal plants (Packovsky and Fuller, 1986; Raju *et al.*, 1990; Li *et al.*, 1991a, Gupta and Janardhanan, 1991; Tawaraya *et al.*, 1995). Li *et al.*, (1991) reported that VAM fungi significantly improved the P nutrition of the host plant (white clover). Gupta and Janardhanan (1991) also found significant increase in uptake of P and K by the inoculation of *G. aggregatum.* Tawaraya *ett al.* (1995) studied the effect of two VAM fungi, *G. mosseae* and *Gigaspora margarita* on phosphorus uptake and growth of white clover and onion plants and found increased phosphorus uptake and growth of both plants. Similarly, in the present investigation, all the

treatments significantly increased P uptake by all VAM inoculated *Cymbopogon* spp from soil. Accumulation of phosphorus in shoot tissues was also greatly enhanced.

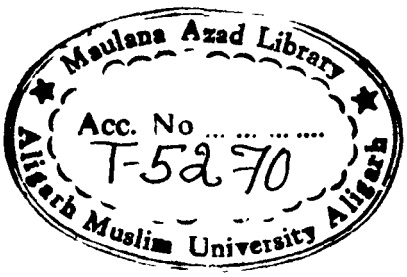
There is increasing evidence of a significant role of VAM in plant N uptake (Johansen *et al.*, 1993, 1996; Bago *et al.*, 1996). Better N content in plant shoot tissues was observed in mycorrhizal plants particularly when inoculated singly with *G. aggregatum* or *G. fasiculatum,* where the increase in N content of plant shoots was maximum as compared to other treatments.

In a number of instances, it has been reported that mycorrhizal plants acquire more N than non-mycorrhizal plants (Smith *et al.*, 1986; Johanson *et al.*, 1993; Frey and Schuepp, 1993; Cliquet *et al.*, 1997). Smith *et al* ., (1986) reported N inflow into the roots of mycorrhizal plants was greatly increased compared to non-mycorrhizal plants. They also noted that under conditions of moderate P defficiency or P sufficiency, mycorrhizal infection improves the ability of plants to absorb more N. Johansen *et al.*, (1993) reported the depletion of soil inorganic N by mycorrhizae. Cliquet *et al.*, (1997) inoculated the *Lolium perenne* with *G. fasciculatum* and observed increased growth and total N content of the plants in all cases.

Increased uptake of K, Zn, Cu, Fe, Co, Ni and Pb by mycorrhizal plants was also reported by Powell (1975), Rhodes and Gerdemann (1978 a, b), Lambert *et al.*, (1979), Gildon and Tinker (1983), Killham and Firestone (1983), Pacovsky (1986), Smith and Roncadori (1986), Raju *et al.*, (1990). Li *et al.*, (1991a,b,c) Sharma and Srivastava (1991), Tawaraya *et al.*, (1995), Al-Karaki and Al-Raddad, (1997). The findings of the present study are similar to these reports. Mycorrhization of *Cymbopogon* spp. enhanced uptake of K, Cu and Zn. The enhance accumulation of K, Cu and Zn might have been because of their increased availabilities i.e; increase uptake from soil by the plants with the help of VAM fungal hyphae.

The current investigation shows that *Cymbopogon* spp. have significant VAM association in nature. Inoculation of these plants with VAM fungi can improve their growth and biomass production. The beneficial effect of VA

mycorrhization is more pronounced in *C. winterianus* than other *Cymbopogon* spp. This is the first attempt to explore the possibility to use VAM fungi for improving growth and productivity of Cymbopogons. The findings of the present study have great economic significance. As several *Cymbopogon* spp. are cultivated on commercial scale, any improvement in their yield would be commercially important. Exploiltation of beneficial effects of VA mycorrhiza would be one of useful methods observed to be employed for improving the crop production of *Cymbopogon* . The current investigation shows great potential of artificial use of VAM fungi for increasing the growth and productivity of *Cymbopogon* used for production of specific essential oils of commercial use.



SUMMARY

Cymbopogon spp. are important essential oil bearing plants. These essential oils have immense commercial value as they are intensively used in perfumeries, cosmetics and pharmaceutical preparations. *C. martinii* (Roxb.) Wats, with two varieties *motia* for the production of plamarosa oil and *sofia* for ginger-grass oil, *C. flexuosus* (Steud.) Wats., *C. citratus* Stapf. and *C. pendulus* Stapf. for lemon-grass oil, *C. winterianus* Jowitt. and *C. nardus* (L.) Rendle for citronella oil are important species of *Cymbopogon* in this respect.

In India, *C. martinii, C. flexuosus* and *C. winterianus* are cultivated on large scale for production of their essential oils for commercial purposes. Important constituents of these essential oils are geraniol, citral, citronellal, citronellol and piperitone. Besides, they also contain a large number of minor constituents. These aromatic compounds are used in preparation of perfumes, cosmetics and pharmaceuticals. In view of their immense commercial and medicinal importance their enhanced production is always desirable. The present study was conducted to evaluate the significance and role endophytic mycorrhizae (VAM fungi) in enhancing productivity of *Cymbopogon* spp. including the essential oils of commerce.

Five cultivated species of *Cymbopogon* namely *C. caesius, C. flexuosus, C. martinii, C. pendulus and C. winterianus* were examined while growing in the fields to find out VAM association. Seasonal variation with regard to level of VAM association was also determined. The five speices of

Cymbopogon grown in the CIMAP experimental farm were sarveyed for VAM association during June 1992 to May 1993 at monthly intervals. All *Cymbopogon* spp. growing in the field exhibited VAM association. Range of root infection varied in different species of *Cymbopogon*. Spore density in the rhizosphere soil was not found to be correlated with the extent of root colonization. Spore counts in the rhizosphere soils of *C. pendulus, C. flexuosus* and *C. winterianus,* were similar and relatively higher than the other two *Cymbopogon* spp. Lowest spore count was found in rhizosphere of *C. martinii.*

Two genera of the VAM fungi, *Gigaspora* and *Glomus* were found to be associated with the field grown *Cymbopogon* spp. Nine species, *G. aggregatum, G. dimorphicum, G. fasiculatum, G. geosporum, G. macrocarpum, G. mosseae, G. multicaulis, G. occultum* and *G. reticulatum* comprised the *Glomus. Gigaspora* was represented by a single speices which, however, could not be identified. Among the VAM fungi that colonized *Cymbopogon* spp., *G. aggregatum,* and *G. reticulatum* were the most frequent. *G. macrocarpum* and *G. occultum* were less frequent. Other species showed infrequent occurrence in rhizosphere of *Cymbopogon* spp.

Seasonal variation in spore number of the VAM fungi in the rhizosphere of all the five *Cymbopogon* spp. showed a similar trend. Spore count was low in summer months, tended to increase in high relative humidity and low temperature, and remained almost stable during winters. Highest spore number was observed in February and March. Adverse effect of water logging on spore

count was also observed. Root infection was maximum in summer months. No correlation was found between spore count and extent of root infection.

For glass house experiment, three commercially important *Cymbopogon* spp. namely *C. flexuosus, C. martinii* and *C. winterianus* were slected to study the effect of VAM inoculation on their growth performance. Three *Glomus* species viz, *G. aggregatum, G. fasiculatum* and *G. mosseae* were used for single and combined inoculations.

C. winterianus plants showed best response of the inoculations with the VAM fungi. A singnificant increase in all the considered growth parameters (plant height, number of tillers and fresh shoot weight) occurred. Inoculation of *C. winteranus* enchanced 56-97% shoot fresh weight with an increase in plant height (34-70%) and tillering (87-150%). Inoculated *C. martinii* plants also showed considerable increase in their shoot fresh weight (10-57%). Plant height and tillering also increased ranging from 7 to 23% and 20 to 30%, respectively. Response of *C. flexuosus* plants to the VAM inoculation was retatively poor, as the enhancement in shoot fresh weight was only 9-49%, in plant height 6-26% and in tillering 15-46%.

Single inoculations with *G. aggregatum* and *G. fasciculatum* were more effective for enhanced growth of the plants in comparison to the mixture of the three VAM fungi . Single inoculation with *G. mosseae* was also not very effective in this respect. Extensive root colonization of all the three *Cymbopogon* spp. occurred. The ranges of root colonization by the VAM

fungi were 82-90%. 78-92% and 87-93% in *C. flexuosus*, *C. martinii* and *C. winterianus*, respectively. Root colonization and spore numbers in rhizosphere did not show any correlation. *G. fasciculatum* did not multiply well in the rhizosphere in comparison to other two VAM fungi. The spore number ranged between 7 and 12 spores g^{-1} soil in three *Cymbopogon* spp. Rhizosphere soil of *Cymbopogon* spp. inoculated with *G. aggregatum* had 22 spores g^{-1} soil, with *G. mosseae*, 20-26 spores g^{-1} soil and with the mixture, 17-21 spores g^{-1} soil.

Essential oil content of the *Cymbopogon* spp. showed a marked increase due to inoculation with the VAM fungi. The increase was maximum in *C. martinii* inoculated with *G. aggreatum* (69%). The increase ranged between 6 to 17% in *C. flexuosus*, 31 to 69% in *C. martinii* and 40 to 50% in *C. winterianus* plants. The total production of the essential oils by the plants exhibited appreciable increase, considering the increase in total herb yield of the plants. Main constituent of these essential oils except lemon-grass oil, however, did not show an increase. In *C. flexuosus* (lemon-grass), a significant increase in citral content was found, on inoculation with *G. fasciculatum*, and *G. mosseae* alone and with mixture of the three species.

Nutrient (N,P,K, Cu and Zn) uptake from the soil by all three species considerably improved by application of the VAM fungi. Greater accumulation of the nutrients in shoot tissues of the inoculated *Cymbopogon* plants was also observed which was in consistence with biomass increase. Maximum enhancement of N,P,K uptake from soil occurred in *C. winterianus* plants.

Maximum Cu uptake was observed in *C. flexuosus* whereas uptake of Zn was maximum in *C. martinii.* Enhancement in accumulation of nutrients (N,K, but not P) was highest in *C. martinii* plants inoculated with *G. fasciculatum.* Phosphorus accumulation was greatest in *C. winterianus* plants inoculated with *G. fasciculatum*. However, enhanced acumulation of N,P,K,Cu and Zn occurred in *C. martinii* plants. Similarly, in *C. winterianus* shoots marked enhancement in accumulation of N,P,K,Cu and Zn was observed. *C. flexuosus* also showed greater accumulation of N,P,K,Cu and Zn in shoot tissues.

The findings of the present investigations show that *Cymbopogon* spp. have abundant VAM association in field. Possibly, they have dependency on VAM fungi in natural conditions. Artificial inoculation of some species of *Cymbopogon* with selected VAM fungi improved their plant growth and biomass production, uptake and accumulation of nutrients like N,P,K, Cu and Zn. Essential oil content of the plants considerably increased. The response of the each species of *Cymbopogon* and effects of all the VAM fungi were, however, not same. Nevertheless, over all performance of the plants showed great improvement due to the VAM inoculation. This situation is of great significance for the essential oil production by these plants and may be commercially exploited.

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